STIC Search Report
Biotech-Chem Library

STIC Database Tracking Number: 104683

TO: Ralph J Gitomer **Location: cm1/11D11** **Art Unit: 1651** **Monday, September 29, 2003** **Case Serial Number: 09/868200**	**From: Barb O'Bryen** **Location: Biotech-Chem Library** **CM1-6A05** **Phone: 308-4291** BOB **barbara.obryen@uspto.gov**

Search Notes

STIC SEARCH RESULTS FEEDBACK FORM

Biotech-Chem Library

Questions about the scope or the results of the search? Contact ***the searcher or contact:***

Mary Hale, Information Branch Supervisor
308-4258, CM1-1E01

Voluntary Results Feedback Form

- *I am an examiner in Workgroup:* ☐ *Example: 1610*
- *Relevant prior art **found**, search results used as follows:*
  - ☐ 102 rejection
  - ☐ 103 rejection
  - ☐ Cited as being of interest.
  - ☐ Helped examiner better understand the invention.
  - ☐ Helped examiner better understand the state of the art in their technology.

Types of relevant prior art found:

- ☐ Foreign Patent(s)
- ☐ Non-Patent Literature
  (journal articles, conference proceedings, new product announcements etc.)

- *Relevant prior art **not found:***
  - ☐ Results verified the lack of relevant prior art (helped determine patentability).
  - ☐ Results were not useful in determining patentability or understanding the invention.

Comments:

Drop off or send completed forms to STIC/Biotech-Chem Library CM1 – Circ. Desk

=> fil capl; d iall 1-2
FILE 'CAPLUS' ENTERED AT 09:22:34 ON 29 SEP 2003
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FILE COVERS 1907 - 29 Sep 2003 VOL 139 ISS 14
FILE LAST UPDATED: 28 Sep 2003 (20030928/ED)

This file contains CAS Registry Numbers for easy and accurate substance identification.

L7 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2000:415612 CAPLUS
TITLE: Method and device for the cell-**track**-based examination and cultivation ofcells
INVENTOR(S): **Fuhr, Gunter; Hagedorn, Rolf;** Shirley, Stephen Graham; **Richter, Ekkehard**
PATENT ASSIGNEE(S): Evotec Biosystems Ag, Germany
SOURCE: PCT Int. Appl.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: German
INT. PATENT CLASSIF.:
MAIN: G01N033-483
SECONDARY: C12N005-00
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000036415	A1	20000622	WO 1999-EP9781	19991210
W: JP, US				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
DE 19857692	C1	20000824	DE 1998-19857692	19981214
EP 1144999	A1	20011017	EP 1999-964544	19991210
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				

PRIORITY APPLN. INFO.: DE 1998-19857692 A 19981214
WO 1999-EP9781 W 19991210

ABSTRACT:
The invention relates to a method for the cell-track-based examination of biological cells, according to which the cells (16) are applied to an at least partly structured and/or surface-modified substrate (11) and move across track areas (13, 15) of the surface of the substrate in an adhesive manner such that they generate cell tracks (14a, 14b) which consist of material residues separated by the cells. The cells are then studied on the basis of these tracks. The invention further relates to a method for cultivating cells on

substrates modified in a biocompatible manner whose surfaces are covered in cell tracks.

REFERENCE COUNT: 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD.

REFERENCE(S):
(1) Aetsrn; FR 2743421 A 1997 CAPLUS
(2) Aetsrn; FR 2743421 A 1997 CAPLUS
(3) Becton Dickinson Co; EP 0347210 A 1989 CAPLUS
(4) Becton Dickinson Co; EP 0347210 A 1989 CAPLUS
(5) Univ Louvain; WO 9615223 A 1996 CAPLUS
(6) Univ Louvain; WO 9615223 A 1996 CAPLUS
(7) Zetter, B; US 4359527 A 1982
(8) Zetter, B; US 4359527 A 1982

L7 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 1998:618949 CAPLUS

TITLE: Cell **traces**. Footprints of individual cells during locomotion and adhesion

AUTHOR(S): **Fuhr, Guenter; Richter, Ekkehard;** Zimmermann, Heiko; Hitzler, Hermine; Niehus, Horst; **Hagedorn, Rolf**

CORPORATE SOURCE: Institut Biologie, Humboldt-Universitaet, Berlin, D-10115, Germany

SOURCE: Biological Chemistry (1998), 379(8/9), 1161-1173
CODEN: BICHF3; ISSN: 1431-6730

PUBLISHER: Walter de Gruyter & Co.

DOCUMENT TYPE: Journal

LANGUAGE: English

ABSTRACT:
Animal cells release traces of material onto glass or silicon surfaces during adhesion and migration. This little studied phenomenon is a widespread and normal concomitant of cell migration. The paper introduces the study of such material. The traces can be visualized by different microscopic techniques (e.g. TIRF, IRM, CLSM, AFM, SEM). Cell traces typical for different cell lines (NIH 373 and L929 mouse fibroblasts, mouse macrophages, mouse sarcoma cells and human osteosarcoma cells) are shown and discussed. There are well organized structures such as different linear and nodular elements as well as patches. Traces can extend up to some hundred micrometers from the cell, but the dimensions of the linear elements are in the submicron range. Cell traces are not identical with focal contacts but can include them. A first classification of basic elements is proposed. It allows an estn. of the total vol. and surface in comparison to the donor cell. Higher order structures are discussed and a first insight into the protein compn. of traces produced by mouse fibroblasts is given. Our observations, together with the cell adhesion literature suggest that the amt. of released material, its extent and chem. and structural properties depend on cell type and physiol. as well as other external influences. Cell traces in combination with the adhesion pattern of the donor cell should give information about the activity and physiol. status of individual cells, the mechanisms of cell locomotion and the mol. compn. of the donor cell membrane. The traces might possibly be used as submicron elements for passive elec. characterization and biotechnol. applications.

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=> fil biosis; d iall
FILE 'BIOSIS' ENTERED AT 09:56:38 ON 29 SEP 2003
COPYRIGHT (C) 2003 BIOLOGICAL ABSTRACTS INC.(R)

FILE COVERS 1969 TO DATE.
CAS REGISTRY NUMBERS AND CHEMICAL NAMES (CNs) PRESENT
FROM JANUARY 1969 TO DATE.

RECORDS LAST ADDED: 24 September 2003 (20030924/ED)
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L19 ANSWER 1 OF 1 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1986:182113 BIOSIS
DOCUMENT NUMBER: BR30:93985
TITLE: INTERACTION OF MIGRATING EMBRYONIC CELLS WITH EXTRACELLULAR MATRIX.
AUTHOR(S): **HAY E D**
CORPORATE SOURCE: DEP. ANAT. CELL. BIOL., HARVARD MED. SCH., 25 SHUTTUCK ST., BOSTON, MASS. 02115, USA.
SOURCE: HAEMMERLI, G. AND P. STRAULI (ED.). EXPERIMENTAL BIOLOGY AND MEDICINE, VOL. 10. MOTILITY OF VERTEBRATE CELLS IN CULTURE AND IN THE ORGANISM: MOLECULAR MECHANISMS AND MORPHOLOGIC MANIFESTATIONS; ZURCHER WORKSHOP ON CELL TRAFFIC IN THE DEVELOPING AND ADULT ORGANISM, ZURICH, SWITZERLAND, NOV. 22-24, 1984. IX+266P. S. KARGER AG: BASEL, SWITZERLAND; NEW YORK, N.Y., USA. ILLUS, (1985) 0 (0), 174-193.
CODEN: EXBMAA. ISSN: 0071-3384. ISBN: 3-8055-4135-X.
FILE SEGMENT: BR; OLD
LANGUAGE: English
CONCEPT CODE: General Biology - Symposia, Transactions and Proceedings of Conferences, Congresses, Review Annuals 00520
Cytology and Cytochemistry - Animal *02506
Movement *12100
Sense Organs, Associated Structures and Functions - Physiology and Biochemistry *20004
Developmental Biology - Embryology - General and Descriptive *25502
Tissue Culture, Apparatus, Methods and Media 32500
In Vitro Studies, Cellular and Subcellular 32600
INDEX TERMS: Miscellaneous Descriptors
CORNEAL FIBROBLAST

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=> fil scisearch
FILE 'SCISEARCH' ENTERED AT 10:00:41 ON 29 SEP 2003
COPYRIGHT 2003 THOMSON ISI

FILE COVERS 1974 TO 26 Sep 2003 (20030926/ED)

=> d ibib ab hit
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L20 ANSWER 1 OF 26 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 2001:540282 SCISEARCH
THE GENUINE ARTICLE: 447QN
TITLE: Mammalian cell traces - morphology, molecular composition, artificial guidance and biotechnological relevance as a new type of "bionanotube"
AUTHOR: Zimmermann H; Richter E; Reichle C; Westphal I; Geggier P; Rehn U; Rogaschewski S; Bleiss W; Fuhr G R (Reprint)

CORPORATE SOURCE: Humboldt Univ, Inst Biol, Zentrum Biophys & Bioinformat, Invalidenstr 42, D-10115 Berlin, Germany (Reprint); Humboldt Univ, Inst Biol, Zentrum Biophys & Bioinformat, D-10115 Berlin, Germany; Humboldt Univ, Inst Phys, Lehrstuhl Oberflachenphys & Atomstossprozesse, D-10115 Berlin, Germany; Humboldt Univ, Inst Biol, Lehrstuhl Mol Parasitol, D-10115 Berlin, Germany
COUNTRY OF AUTHOR: Germany
SOURCE: APPLIED PHYSICS A-MATERIALS SCIENCE & PROCESSING, (JUL 2001) Vol. 73, No. 1, pp. 11-26.
Publisher: SPRINGER-VERLAG, 175 FIFTH AVE, NEW YORK, NY 10010 USA.
ISSN: 0947-8396.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 34

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB During locomotion, adherently growing cells release highly ordered structures consisting of filaments: and patches often dendritically organised. Such traces can be re-incorporated by the donor cell or disconnected and lost. Here, we present the results of a three-year research programme into trace formation and structural organisation including the influence of substrate surface properties. Some phenomena may, ultimately, have medical or technological applications. These include: (i) the deposition and re-incorporation of cellular material as cells move forward and backward; (ii) the ability of cells to differentiate between their own and foreign traces; (iii) the presence of receptors in the intact membrane envelope of filaments and patches; and (iv) the cytoplasmic content of patches. Trace formation is physiologically controlled and a characteristic of many types of actively migrating higher animal and human cells. Possible applications and perspectives are discussed and the importance of cell-trace elements as "bionanotubes" and biological submicron compartments of cells is explained.

Referenced Author (RAU)	Year (RPY)	VOL (RVL)	PG (RPG)	Referenced Work (RWK)	
HAY E D	1985	10	174	EXP BIOL MED	<--

=> d ibib ab hit 2-26

L20 ANSWER 2 OF 26 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 2001:151343 SCISEARCH
THE GENUINE ARTICLE: 397YN
TITLE: Altered trophoblastic differentiation and increased trophoblastic invasiveness during delayed development in the short-tailed fruit bat, Carollia perspicillata
AUTHOR: Badwaik N K; Rasweiler J J (Reprint)
CORPORATE SOURCE: Cornell Univ, Weill Med Coll, Dept Obstet & Gynecol, 1300 York Ave, New York, NY 10021 USA (Reprint); Cornell Univ, Weill Med Coll, Dept Obstet & Gynecol, New York, NY 10021 USA
COUNTRY OF AUTHOR: USA
SOURCE: PLACENTA, (JAN 2001) Vol. 22, No. 1, pp. 124-144.
Publisher: W B SAUNDERS CO LTD, 24-28 OVAL RD, LONDON NW1 7DX, ENGLAND.
ISSN: 0143-4004.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 38

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB During pregnancy in the short-tailed fruit bat, lengthy

post-implantational delays in conceptus development can occur in response to stress in captivity and seasonally in the wild. When comparisons were made between uteri carrying embryos in delay at the primitive streak stage and those growing more rapidly, many differences were noted. During delay the developing chorioallantoic placenta was generally smaller, contained a higher ratio of cytotrophoblast to syncytiotrophoblast, and had been invaded only to a limited er;tent on its embryonic side by mesoderm. Furthermore, much of the cytotrophoblast appeared relatively undifferentiated, randomly-oriented, linked primarily by primitive junctions, and lacked a basal lamina. In contrast, in placentae serving somite and limb-bud stage embryos, sizeable areas were noted that consisted only of more highly differentiated syncytiotrophoblast perforated by maternal vascular spaces (trophospongium). The first contact of the allantois with the developing placenta was also noted at the somite stage, and this initiated widespread invasion of the placenta by mesenchyme and allantoic blood vessels. Wherever this invasion had occurred, the cytotrophoblast between the mesenchyme and syncytiotrophoblast of the interhaemal barrier consisted of a single, polarized layer of more differentiated cells with an associated basal lamina. Eventually, all of the trophospongium was invaded by cytotrophoblast and vascularized fetal mesenchyme. These observations suggest that in addition to its germinal function, cytotrophoblast in this bat may play a major role in controlling mesenchymal invasion and angiogenesis on the embryonic side of the placenta. During the period of delay, highly invasive trophoblast is also released by the placenta. This invades the myometrium and sometimes extrauterine tissues via intcrstitial migration along maternal capillaries and veins. to 2001 Harcourt Publishers Ltd.

Referenced Author (RAU)	Year (RPY)	VOL (RVL)	PG (RPG)	Referenced Work (RWK)
HAY E D	1985	10	174	EXP BIOL MED <--

L20 ANSWER 3 OF 26 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 2001:138396 SCISEARCH
THE GENUINE ARTICLE: 398ED
TITLE: A novel approach to studying the migratory morphology of embryonic mesenchymal cells
AUTHOR: Harkin D G (Reprint); Bianco J I; Collins N
CORPORATE SOURCE: Griffith Univ, Sch Biomol & Biomed Sci, Nathan, Qld 4111, Australia (Reprint)
COUNTRY OF AUTHOR: Australia
SOURCE: BIOLOGY OF THE CELL, (OCT 2000) Vol. 92, No. 7, pp. 537-543.
Publisher: EDITIONS SCIENTIFIQUES MEDICALES ELSEVIER, 23 RUE LINOIS, 75724 PARIS CEDEX 15, FRANCE.
ISSN: 0248-4900.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 29

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A polarized morphology, defined by extension of an anterior pseudopod, is essential for the amoeboid migration of embryonic mesenchymal cells. Leukocytes adopt a similar morphology immediately following suspension in simple buffers containing chemotactic factors. Polarization in suspension therefore provides a rapid and sensitive screening assay for putative regulators of leukocyte migration. The aim of the present study was to investigate whether this assay might also be used to study the motile behaviour of embryonic mesenchymal cells. Primary cultures of mesenchymal cells were established from explants of stage 28 chick embryo corneal-limbal stroma. Serum-starved, subconfluent cultures were harvested using ethylenediamine tetra-acetic acid and resuspended in Hanks' solution for up to 15 min at 37 degreesC. A variety of cell shapes, including

spherical cells, blebbed cells, and cells with either non-polarized or polarized pseudopodia were observed. The proportions of cells with pseudopodia increased significantly over time. Treatment of cells with the chemotactic mitogen platelet derived growth factor-BE (PDGF-BB, homodimer isoform) suppressed blebbing and increased both pseudopod formation and polarization. Optimal polarization occurred in concentrations of PDGF-BB that are similar to those required for optimal chemotaxis (10 ng.mL(-1)). The polarization observed in the absence of PDGF-BB suggests that the migration of cells examined in this study might be controlled at least in part by some intrinsic mechanism. In addition, the strong polarization response to PDGF-BB confirms the role for this growth factor during corneal development. Observations of mesenchymal cell morphology in suspension, therefore provide novel data regarding the motile behaviour of embryonic cells. (C) 2000 Editions scientifiques et medicales Elsevier SAS.

Referenced Author (RAU)	Year (RPY)	VOL (RVL)	PG (RPG)	Referenced Work (RWK)	
HAY E D	1985	10	174	EXP BIOL MED	<--

L20 ANSWER 4 OF 26 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 2000:745744 SCISEARCH
THE GENUINE ARTICLE: 358NA
TITLE: Trace formation during locomotion of L929 mouse fibroblasts continuously recorded by interference reflection microscopy (IRM)
AUTHOR: Richter E (Reprint); Hitzler H; Zimmermann H; Hagedorn R; Fuhr G
CORPORATE SOURCE: HUMBOLDT UNIV, INST BIOL, INST MEMBRANPHYSIOL, INVALIDENSTR 42, D-10115 BERLIN, GERMANY (Reprint)
COUNTRY OF AUTHOR: GERMANY
SOURCE: CELL MOTILITY AND THE CYTOSKELETON, (SEP 2000) Vol. 47, No. 1, pp. 38-47.
Publisher: WILEY-LISS, DIV JOHN WILEY & SONS INC, 605 THIRD AVE, NEW YORK, NY 10158-0012.
ISSN: 0886-1544.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 17

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The recently reported formation of highly ordered traces by migrating cells has been studied on L929 fibroblasts in time lapse experiments by means of interference reflection microscopy (IRM) as well as by conventional microscopy. Formation of pronounced traces on glass substrates correlates to migration after cell division, and the trace arrangement on the substrate depends on migration velocity: slow migration results in a highly branched, broad, and relatively short trace, while fast migration yields a slim and long trace with few branches. IRM-irradiation caused cessation of locomotion and trace formation and accelerated degradation of existing traces. Traces consist of cord-like cytoplasmic strands, which contain F-actin filaments and they seem to be enveloped by a membrane. It is supposed that cell traces are homologous to filopodia. Traces arise mainly from non-retracted filopodia at the rear margin of the migrating cell. The branches within the traces are the result of the repeated stretching out of a backwardly directed lamellipodium. They arise from the formation of new filopodia that emerge at the actin ribs of the lamellipodium. (C) 2000 Wiley-Liss, Inc.

Referenced Author (RAU)	Year (RPY)	VOL (RVL)	PG (RPG)	Referenced Work (RWK)	
HAY E D	1985	10	174	EXP BIOL MED	<--

L20 ANSWER 5 OF 26 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 2000:25848 SCISEARCH
THE GENUINE ARTICLE: 270CQ
TITLE: Effect of cell migration on the maintenance of tension on a collagen matrix
AUTHOR: Roy P; Petroll W M (Reprint); Chuong C J; Cavanagh H D; Jester J V
CORPORATE SOURCE: UNIV TEXAS, SW MED CTR, DEPT OPHTHALMOL, 5323 HARRY HINES BLVD, DALLAS, TX 75235 (Reprint); UNIV TEXAS, SW MED CTR, DEPT OPHTHALMOL, DALLAS, TX 75235; UNIV TEXAS, SW MED CTR, JOINT PROGRAM BIOMED ENGN, DALLAS, TX 75230; UNIV TEXAS, ARLINGTON, TX 76019
COUNTRY OF AUTHOR: USA
SOURCE: ANNALS OF BIOMEDICAL ENGINEERING, (NOV-DEC 1999) Vol. 27, No. 6, pp. 721-730.
Publisher: AMER INST PHYSICS, CIRCULATION FULFILLMENT DIV, 500 SUNNYSIDE BLVD, WOODBURY, NY 11797-2999.
ISSN: 0090-6964.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 42

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Although it is known that cells promote structural reorganization of the collagen architecture, how individual cells exert mechanical tension on the matrix is not clearly understood. In the present study we have investigated the mechanical interaction of individual corneal fibroblasts with a collagen matrix using an improved version of our previously described in vitro force-measurement system (Roy, P. et al. Exp. Cell Res. 232:106-117, 1997). The elastic distortion of the collagen matrix exerted by cells was temporally recorded and analyzed using a two-dimensional finite-element model to quantify the forces exerted on the matrix. Time-lapse videomicroscopy of serum-cultured cells on the matrix for up to 6 h revealed that individual fibroblasts generated measurable tension on the matrix during pseudopodial extension and slow retraction. Fast retraction, an event observed during active cell migration, was associated with dramatic release of tension on the matrix. An apparent inverse correlation was observed between cell translocation and maintenance of matrix tension. Additional experiments with cells under serum-free conditions revealed that these cells fail to generate any detectable tension on the matrix despite undergoing filopodial extension and retraction. Since serum-free cells do not form focal adhesions or stress fibers, these experimental data suggest that contractility of nonmotile cells, coupled with strong cell-matrix adhesion, is the most favorable mechanism of generating and maintaining tension on the extracellular matrix. (C) 1999 Biomedical Engineering Society. [S0090-6964(99)00106-X].

Referenced Author (RAU)	Year (RPY)	VOL (RVL)	PG (RPG)	Referenced Work (RWK)	
HAY E D	1985	10	174	EXP BIOL MED	<--

L20 ANSWER 6 OF 26 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 2000:25790 SCISEARCH
THE GENUINE ARTICLE: 270CJ
TITLE: Kinetic model for integrin-mediated adhesion release during cell migration
AUTHOR: Palecek S P (Reprint); Horwitz A F; Lauffenburger D A
CORPORATE SOURCE: UNIV CHICAGO, CTR MOL ONCOL, JULES F KNAPP CTR, ROOM R322, 924 E 57TH ST, CHICAGO, IL 60637 (Reprint); MIT, DEPT CHEM ENGN, CAMBRIDGE, MA 02139; UNIV ILLINOIS, DEPT CELL & STRUCT BIOL, URBANA, IL 61801; MIT, DIV BIOENGN & ENVIRONM HLTH, CAMBRIDGE, MA 02139; MIT, CTR BIOMED ENGN, CAMBRIDGE, MA 02139

COUNTRY OF AUTHOR: USA
SOURCE: ANNALS OF BIOMEDICAL ENGINEERING, (MAR-APR 1999) Vol. 27, No. 2, pp. 219-235.
Publisher: AMER INST PHYSICS, CIRCULATION FULFILLMENT DIV, 500 SUNNYSIDE BLVD, WOODBURY, NY 11797-2999.
ISSN: 0090-6964.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 47

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Under many circumstances, cell migration speed is limited by the rate of cell-substratum detachment at the cell rear. We have constructed a mathematical model to integrate how the biophysical and biochemical interactions between integrins, the cytoskeleton, and the matrix affect rear retraction and linkage dissociation mechanisms. Our model also examines how applied forces and integrin clustering affect retraction kinetics. The model predicts two distinct detachment phenotypes. In the first, detachment is extremely rapid, dominated by integrin extracellular-matrix dissociation, and it occurs at high forces or low adhesiveness. In the second, detachment is much slower, dominated by integrin-cytoskeleton dissociation, and it occurs at low forces or high adhesiveness. The amount of integrin extracted from the rear of the cell is an assay for the detachment phenotype. During rapid detachment cells leave little integrin on the substratum whereas during slow detachment a large fraction of integrin rips from the membrane. This model delineates parameters which can be exploited to regulate cell speed in each detachment regime. The model also offers an explanation as to why some cell types, such as leukocytes or keratocytes, are able to detach easily and move very quickly while other cell types, such as fibroblasts, tend to migrate more slowly and release many more integrins during detachment. (C) 1999 Biomedical Engineering Society. [S0090-6964(99)02302-4].

Referenced Author (RAU)	Year (RPY)	VOL (RVL)	PG (RPG)	Referenced Work (RWK)
HAY E D	1985	10	174	EXP BIOL MED <--

L20 ANSWER 7 OF 26 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 1999:695561 SCISEARCH
THE GENUINE ARTICLE: 233LQ
TITLE: Topography of cell traces studied by atomic force microscopy
AUTHOR: Zimmermann H; Hagedorn R; Richter E; Fuhr G (Reprint)
CORPORATE SOURCE: HUMBOLDT UNIV, MATH NAT WISSENSCH FAK 1, INST BIOL, LEHRSTUHL MEMBRANPHYSIOL, INVALIDENSTR 42, D-10115 BERLIN, GERMANY (Reprint); HUMBOLDT UNIV, MATH NAT WISSENSCH FAK 1, INST BIOL, LEHRSTUHL MEMBRANPHYSIOL, D-10115 BERLIN, GERMANY
COUNTRY OF AUTHOR: GERMANY
SOURCE: EUROPEAN BIOPHYSICS JOURNAL WITH BIOPHYSICS LETTERS, (SEP 1999) Vol. 28, No. 6, pp. 516-525.
Publisher: SPRINGER VERLAG, 175 FIFTH AVE, NEW YORK, NY 10010.
ISSN: 0175-7571.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 33

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Migrating adherent cells release material onto artificial substrates like glass and silicon while moving. Traces of mouse fibroblasts (L929) have been visualised by atomic force microscopy (AFM). ''Non-contact'' mode AFM in a liquid environment can extract topographic information from

these traces. This dynamic mode allows the study of these soft structures without damage or compression. The AFM images show crossing and branching networks (with specific angles of branching), structured patches, nodular elements, linear elements with irregular height and other features. Fourier analysis of segment spacing in the strands is presented. These spatial features of fibroblast traces are strong indications that actin linked to structural proteins is involved in the formation of cell traces. We also give methods for trace preparation and undistorted imaging and discuss further perspectives.

Referenced Author (RAU)	Year (RPY)	VOL (RVL)	PG (RPG)	Referenced Work (RWK)	
HAY E D	1985	10	174	EXP BIOL MED	<--

L20 ANSWER 8 OF 26 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 1998:932382 SCISEARCH
THE GENUINE ARTICLE: 144TZ
TITLE: Regulation of integrin-mediated adhesion during cell migration
AUTHOR: Cox E A (Reprint); Huttenlocher A
CORPORATE SOURCE: UNIV ILLINOIS, DEPT CELL & STRUCT BIOL, CHEM & LIFE SCI LAB B107, 601 S GOODWIN AVE, URBANA, IL 61801 (Reprint)
COUNTRY OF AUTHOR: USA
SOURCE: MICROSCOPY RESEARCH AND TECHNIQUE, (1 DEC 1998) Vol. 43, No. 5, pp. 412-419.
Publisher: WILEY-LISS, DIV JOHN WILEY & SONS INC, 605 THIRD AVE, NEW YORK, NY 10158-0012.
ISSN: 1059-910X.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 77

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Migrating cells form dynamic and highly regulated adhesive interactions with their environment. In particular, integrin-mediated adhesions to the extracellular matrix (ECM) play a central role in cell migration. This review focuses on recent advances in understanding the adhesive mechanisms that regulate cell detachment at the rear of migrating fibroblasts and neutrophils. The contribution of several key adhesive regulators is discussed, including myosin mediated cell contractility, tyrosine phosphorylation, rho, calcium fluxes, and calpain. A challenge for future investigation will be to determine how adhesive events are spatially and temporally coordinated to promote productive directional cell movements. (C) 1998 Wiley-Liss, Inc.

Referenced Author (RAU)	Year (RPY)	VOL (RVL)	PG (RPG)	Referenced Work (RWK)	
HAY E D	1985	10	174	EXP BIOL MED	<--

L20 ANSWER 9 OF 26 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 1998:745322 SCISEARCH
THE GENUINE ARTICLE: 122NJ
TITLE: Cell traces - Footprints of individual cells during locomotion and adhesion
AUTHOR: Fuhr G (Reprint); Richter E; Zimmermann H; Hitzler H; Niehus H; Hagedorn R
CORPORATE SOURCE: HUMBOLDT UNIV, INST BIOL, INVALIDENSTR 42, D-10115 BERLIN, GERMANY (Reprint); HUMBOLDT UNIV, INST PHYS, D-10115 BERLIN, GERMANY
COUNTRY OF AUTHOR: GERMANY
SOURCE: BIOLOGICAL CHEMISTRY, (AUG-SEP 1998) Vol. 379, No. 8-9, pp. 1161-1173.
Publisher: WALTER DE GRUYTER & CO, GENTHINER STRASSE 13,

D-10785 BERLIN, GERMANY.
ISSN: 1431-6730.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 34

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Animal cells release traces of material onto glass or silicon surfaces during adhesion and migration, This little studied phenomenon is a widespread and normal concomitant of cell migration, The paper introduces the study of such material. The traces can be visualised by different microscopic techniques (e.g. TIRF, IRM, CLSM, AFM, SEM). Cell traces typical for different cell lines (NIH 3T3 and L929 mouse fibroblasts, mouse macrophages, mouse sarcoma cells and human osteosarcoma cells)are shown and discussed, There are well organised structures such as different linear and nodular elements as well as patches. Traces can extend up to some hundred micrometers from the cell, but the dimensions of the linear elements are in the submicron range, Cell traces are not identical with focal contacts but can include them, A first classification of basic elements is proposed, It allows an estimation of the total volume and surface in comparison to the donor cell, Higher order structures are discussed and a first insight into the protein composition of traces produced by mouse fibroblasts is given. Our observations, together with the cell adhesion literature suggest that the amount of released material, its extent and chemical and structural properties depend on cell type and physiology as well as other external influences. Cell traces in combination with the adhesion pattern of the donor cell should give information about the activity and physiological status of individual cells, the mechanisms of cell locomotion and the molecular composition of the donor cell membrane, The traces might possibly be used as submicron elements for passive electric characterisation and biotechnological applications.

Referenced Author (RAU)	Year (RPY)	VOL (RVL)	PG (RPG)	Referenced Work (RWK)	
HAY E D	1985	10	174	EXP BIOL MED	<--

L20 ANSWER 10 OF 26 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 1998:355783 SCISEARCH
THE GENUINE ARTICLE: ZL530
TITLE: Physical and biochemical regulation of integrin release during rear detachment of migrating cells
AUTHOR: Palecek S P; Huttenlocher A; Horwitz A F; Lauffenburger D A (Reprint)
CORPORATE SOURCE: MIT, DEPT CHEM ENGN, CAMBRIDGE, MA 02139 (Reprint); MIT, DEPT CHEM ENGN, CAMBRIDGE, MA 02139; MIT, CTR BIOMED ENGN, CAMBRIDGE, MA 02139; UNIV ILLINOIS, DEPT CELL & STRUCT BIOL, URBANA, IL 61801; UNIV ILLINOIS, DEPT PEDIAT, URBANA, IL 61801
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF CELL SCIENCE, (APR 1998) Vol. 111, Part 7, pp. 929-940.
Publisher: COMPANY OF BIOLOGISTS LTD, BIDDER BUILDING CAMBRIDGE COMMERCIAL PARK COWLEY RD, CAMBRIDGE, CAMBS, ENGLAND CB4 4DL.
ISSN: 0021-9533.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 56

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Cell migration can be considered as a repeated cycle of membrane protrusion and attachment, cytoskeletal contraction and rear detachment,

At intermediate and high levels of cell-substratum adhesiveness, cell speed appears to be rate-limited by rear detachment, specifically by the disruption of cytoskeleton-adhesion receptor-extracellular matrix (ECM) linkages. Often, cytoskeletal linkages fracture to release integrin adhesion receptors from the cell. Cell-extracellular matrix bonds may also dissociate, allowing the integrins to remain with the cell, To investigate molecular mechanisms involved in fracturing these linkages and regulating cell speed? we have developed an experimental system to track integrins during the process of rear retraction in Chinese hamster ovary (CHO) cells, Integrin expression level was varied by transfecting CHO B2 cells, which express very little endogenous alpha 5 integrin, with a plasmid containing human alpha 5 integrin cDNA and sorting the cells into three populations with different alpha 5 expression levels, Receptor/ligand affinity was varied using CHO cells transfected with either alpha IIb beta 3 or alpha IIb beta 3(beta 1-2), a high affinity variant. alpha IIb beta 3(beta 1-2) is activated to a higher affinity state with an anti-LIBS2 antibody. Fluorescent probes were conjugated to non-adhesion perturbing antiintegrin antibodies, which label integrins in CHO cells migrating on a matrix-coated glass coverslip, The rear retraction area was determined using phase contrast microscopy and integrins initially in this area were tracked by fluorescence microscopy and a cooled CCD camera.

We find that rear retraction rate appears to limit cell speed at intermediate and high adhesiveness, but not at low adhesiveness. Upon rear retraction, the amount of integrin released from the cell increases as extracellular matrix concentration, receptor level and receptor-ligand affinity increase, In fact, integrin release is a constant function of cell-substratum adhesiveness and the number of cell-substratum bonds. In the adhesive regime where rear detachment limits the rate of cell migration, cell speed has an inverse relationship to the amount of integrin released at the rear of the cell, At high cell-substratum adhesiveness, calpain, a Ca2+-dependent protease, is also involved in release of cytoskeletal linkages during rear retraction. Inhibition of calpain results in decreased integrin release from the cell membrane, and consequently a decrease in cell speed, during migration, These observations suggest a model for rear retraction in which applied tension and calpain-mediated cytoskeletal linkage cleavage are required at high adhesiveness, but only applied tension is required at low adhesiveness.

Referenced Author (RAU)	Year (RPY)	VOL (RVL)	PG (RPG)	Referenced Work (RWK)	
HAY E D	1985	10	174	EXP BIOL MED	<--

L20 ANSWER 11 OF 26 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 1998:220754 SCISEARCH
THE GENUINE ARTICLE: ZB324
TITLE: Immunolocalisation of collagens in the developing rat molar tooth
AUTHOR: Webb P P; Moxham B J (Reprint); Ralphs J R; Benjamin M
CORPORATE SOURCE: UNIV WALES COLL CARDIFF, SCH MOL & MED BIOSCI, ANAT UNIT, CARDIFF CF1 3US, S GLAM, WALES (Reprint); UNIV WALES COLL CARDIFF, SCH MOL & MED BIOSCI, ANAT UNIT, CARDIFF CF1 3US, S GLAM, WALES
COUNTRY OF AUTHOR: WALES
SOURCE: EUROPEAN JOURNAL OF ORAL SCIENCES, (JAN 1998) Vol. 106, Supp. [1], pp. 147-155.
Publisher: MUNKSGAARD INT PUBL LTD, 35 NORRE SOGADE, PO BOX 2148, DK-1016 COPENHAGEN, DENMARK.
ISSN: 0909-8836.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE; CLIN
LANGUAGE: English
REFERENCE COUNT: 57
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB This study identifies different types of collagens during tooth development, maturation and ageing. Tissues from the rat first molar (from animals ranging in age from E14 to 104 wk postnatally) were immunostained using a panel of mono-and polyclonal antibodies against types I, II, III, IV, VI, IX and X collagen, fibronectin and laminin. During tooth development, types I and III collagens were expressed in the dental papilla at all stages but were also unexpectedly observed in the stellate reticulum of the enamel organ. Transient expression of type II collagen was also observed in the stellate reticulum during the late bell stage. Types IV and VI collagens, with laminin and fibronectin, were located within the basement membranes of the tooth germ. Collagen types I and III were observed within the developing follicle/periodontal ligament, type III predominating where collagen fibres were inserting into the alveolar bone and cementum. The pattern of types I and III collagen labelling within the periodontal ligament and the dental pulp did not change with age. Thus, some unusual collagen localisations were observed in the tooth germ, particularly within the stellate reticulum.

Referenced Author (RAU)	Year (RPY)	VOL (RVL)	PG (RPG)	Referenced Work (RWK)	
HAY E D	1985	10	174	EXP BIOL MED	<--

L20 ANSWER 12 OF 26 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 97:326344 SCISEARCH
THE GENUINE ARTICLE: WV162
TITLE: An in vitro force measurement assay to study the early mechanical interaction between corneal fibroblasts and collagen matrix
AUTHOR: Roy P; Petroll W M; Cavanagh H D; Chuong C J; Jester J V (Reprint)
CORPORATE SOURCE: UNIV TEXAS, SW MED CTR, DEPT OPHTHALMOL, 5323 HARRY HINES BLVD, DALLAS, TX 75235 (Reprint); UNIV TEXAS, SW MED CTR, DEPT OPHTHALMOL, DALLAS, TX 75235; UNIV TEXAS, SW MED CTR, JOINT PROGRAM BIOMED ENGN, DALLAS, TX 75235; UNIV TEXAS, ARLINGTON, TX 76019
COUNTRY OF AUTHOR: USA
SOURCE: EXPERIMENTAL CELL RESEARCH, (10 APR 1997) Vol. 232, No. 1, pp. 106-117.
Publisher: ACADEMIC PRESS INC JNL-COMP SUBSCRIPTIONS, 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495.
ISSN: 0014-4827.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 46

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB An in vitro force measurement assay has been developed to quantify the forces exerted by single corneal fibroblasts during the early interaction with a collagen matrix. Corneal fibroblasts were sparsely seeded on top of collagen matrices whose stiffness was predetermined by micromanipulation with calibrated fine glass microneedles. The forces exerted by individual cells were calculated from time-lapse videomicroscopic recordings of the 2-D elastic distortion of the matrix. In additional experiments, the degree of permanent reorganization of the collagen matrices was assessed by lysing the cells with 1% Triton X-100 solution at the end of a S-hour incubation and recording the subsequent relaxation. The data suggest that a cell can exert comparable centripetal force during either extension of a cell process or partial retraction of an extended pseudopodia. The rates of force associated with pseudopodial extension and partial retraction were 0.180 +/- 0.091 (x10(-8)) N/min (n = 8 experiments) and 0.213 +/- 0.063 (x10(-8)) N/min (It = 8 experiments), respectively. Rupture of pseudopodial adhesion associated with cell locomotion causes a release of force on the matrix and a complete recoil of the pseudopodia concerned; a

simultaneous release of force on the matrix was also observed at the opposite end of the cell. Lysis of cells resulted in 84 +/- 18% relaxation of the matrix, suggesting that little permanent remodeling of matrix is produced by the actions of isolated migrating cells. (C) 1997 Academic Press.

Referenced Author (RAU)	Year (RPY)	VOL (RVL)	PG (RPG)	Referenced Work (RWK)	
HAY E D	1985	10	174	EXP BIOL MED	<--

L20 ANSWER 13 OF 26 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 96:898097 SCISEARCH
THE GENUINE ARTICLE: VV843
TITLE: Effects of electroporation on the tubulin cytoskeleton and directed migration of corneal fibroblasts cultured within collagen matrices
AUTHOR: Harkin D G (Reprint); Hay E D
CORPORATE SOURCE: HARVARD UNIV, SCH MED, DEPT CELL BIOL, 220 LONGWOOD AVE, BOSTON, MA 02115 (Reprint)
COUNTRY OF AUTHOR: USA
SOURCE: CELL MOTILITY AND THE CYTOSKELETON, (NOV-DEC 1996) Vol. 35, No. 4, pp. 345-357.
Publisher: WILEY-LISS, DIV JOHN WILEY & SONS INC 605 THIRD AVE, NEW YORK, NY 10158-0012.
ISSN: 0886-1544.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 49

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Electroporation provides a useful method for loading fibroblasts with fluorescent probes for the cytoskeleton, but the possible deleterious effects of this loading technique on cell motility are unknown. We have used conventional and confocal microscopy of living cells and immunohistochemistry to examine the migration and cytoskeleton of chick embryo corneal fibroblasts electroporated while cultured within collagen gels. Fibroblasts cultured in collagen (1 mg/ml) are successfully electroloaded (0.5-1.0 kV cm(-1)/960 mu F in DMEM/F12/20 mM Hepes, pH 7.2) with dextran (4-150 kDa) and immunoglobulin, but subsequently display uncoordinated pseudopodia and hence are unable to migrate effectively in any one direction. The lack of directed movement is due to depolymerization of microtubules and/or a perinuclear collapse of vimentin filaments, seemingly caused by millimolar levels of Ca2+ ions derived from culture medium following electroporation. Fibroblasts loaded in a buffer which resembles intracellular fluid (less than or equal to 10 mu M Ca2+) maintain their cytoskeleton and continue to migrate, when returned to culture medium within 10 min. Using this novel approach, we have loaded fibroblasts migrating through extracellular matrix (ECM) with rhodamine phalloidin and monitored the behavior of the labeled actin cortex by confocal microscopy. During migration phalloidin-actin accumulates near the base of pseudopodia and at the rear of the cell where it is subsequently left behind. We conclude that electroporation is a valuable technique for loading fibroblasts to study migration within ECM, provided that the conditions used support stability of the tubulin cytoskeleton. (C) 1996 Wiley-Liss, Inc.

Referenced Author (RAU)	Year (RPY)	VOL (RVL)	PG (RPG)	Referenced Work (RWK)	
HAY E D	1985	10	174	EXP BIOL MED	<--

L20 ANSWER 14 OF 26 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 96:420451 SCISEARCH
THE GENUINE ARTICLE: UN100

TITLE: INTEGRIN DYNAMICS ON THE TAIL REGION OF MIGRATING FIBROBLASTS
AUTHOR: PALECEK S P; SCHMIDT C E; LAUFFENBURGER D A (Reprint); HORWITZ A F
CORPORATE SOURCE: MIT, DEPT CHEM ENGN, CAMBRIDGE, MA, 02139 (Reprint); MIT, DEPT CHEM ENGN, CAMBRIDGE, MA, 02139; UNIV ILLINOIS, DEPT CHEM ENGN, URBANA, IL, 61801; MIT, CTR BIOMED ENGN, CAMBRIDGE, MA, 02139; UNIV ILLINOIS, DEPT CELL & STRUCT BIOL, URBANA, IL, 61801
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF CELL SCIENCE, (MAY 1996) Vol. 109, Part 5, pp. 941-952.
ISSN: 0021-9533.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 39

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Cell migration is a complex process that can be considered as a repeated cycle of lamellipod extension and attachment, cytoskeletal contraction, and tail detachment, While lamellipodial and cytoskeletal phenomena are currently the focus of considerable research on cell migration, under many conditions locomotion appears to be rate-limited by events at the cell rear, especially release of cell/substratum adhesions, To study the mechanism of tail detachment, we have developed a novel experimental system that permits observation of integrin dynamics on the ventral surface of migrating fibroblasts. Photoactivatable caged fluorescein is to a non-adhesion-perturbing anti-avian-beta 1 subunit antibody, which labels integrins on chicken fibroblasts migrating on a laminin-coated glass coverslip, Ultraviolet light is focused through a pinhole to photoactivate the caged fluorophore in a 10-mu m-diameter spot at the rear of a polarized cell, The fate of integrins initially present in this spot is monitored using a cooled CCD camera to follow the movement of fluorescent intensity as a function of time over a 2 to 3 hour period. We find that a substantial fraction of the integrins is left behind on the substratum as the cell detaches and locomotes, while another fraction collects into vesicles which are transported along the cell body as the cell migrates, As aggregates rip from the cell membrane, the integrin-cytoskeletal bonds are preferentially fractured resulting in 81+/-15% of the integrin remaining attached to the substratum, We additionally find that adhesions sometimes disperse into integrins which can form new adhesions at other locations in the cell, Adhesions along the cell edge can release from the substrate and translocate with the cell, They either disperse in the cell membrane, rip from the cell membrane and remain attached to the substratum, or form a new aggregate, These observations indicate that the behavior of integrins at the cell rear is much more dynamic than previously appreciated, suggesting that an important locus for regulation of motility may reside in this region.

Referenced Author (RAU)	Year (RPY)	VOL (RVL)	PG (RPG)	Referenced Work (RWK)	
HAY E D	1985	10	174	EXP BIOL MED	<--

L20 ANSWER 15 OF 26 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 96:290433 SCISEARCH
THE GENUINE ARTICLE: UE109
TITLE: AN OVERVIEW OF EPITHELIOMESENCHYMAL TRANSFORMATION
AUTHOR: HAY E D (Reprint)
CORPORATE SOURCE: HARVARD UNIV, SCH MED, DEPT CELL BIOL, 220 LONGWOOD AVE, BOSTON, MA, 02115 (Reprint)
COUNTRY OF AUTHOR: USA
SOURCE: ACTA ANATOMICA, (1995) Vol. 154, No. 1, pp. 8-20.
ISSN: 0001-5180.

DOCUMENT TYPE: General Review; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 101

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Epithelium is the tissue phenotype of early embryos and primitive adults of the chordate phylum. A second tissue type, however, is produced by epithelial-mesenchymal transformation (EMT) in higher chordates, such as vertebrata. Mesenchymal cells have the ability, which true epithelia do not, to invade and migrate through the extracellular matrix (ECM) to create dramatic cell transpositions. The first-formed or primary mesenchymal cells in amniote vertebrates migrate from the primitive streak to differentiate into the mesodermal and endodermal epithelia. Definitive mesenchyme with connective tissue and muscle potentials arises from the epithelial mesoderm at about the same time as the neural crest mesenchyme forms from the ectoderm. Later on in embryogenesis. EMT is used to remodel unwanted epithelia, such as that of the palate medial edges. We discuss the mechanisms by which epithelial cells transform into mesenchyme and vice versa. On the one hand, cells activate putative mesenchymal master genes, turn off epithelial genes, and acquire motility machinery that allows them to interact in 3 dimensions (3D) with ECM via actin cortex while sliding their endoplasm into their new front ends. On the other hand, primary mesenchymal cells can reactivate epithelial regulatory genes, such as E-cadherin, turn off the motility machinery for invading ECM, and reexpress apical-basal polarity. We review the genes, such as FSP1, src, ras, and fos, that are activated in cells transforming to mesenchyme and the genes their neighbors activate to induce EMT, such as those for TGF beta, NT-3, and sonic hedgehog. Suspension in 3D collagen gels can induce adult epithelium to undergo EMT; alpha 5 beta 1 integrin is activated on surfaces in contact with collagen, including apical surfaces that do not normally express integrins. In vivo, it is possible that pathological manipulations of a cell's environment likewise induce EMT. Of the examples we give, the creation of invasive metastatic carcinoma cells by EMT is the most fearful. Interestingly, transfection of either metastatic cells or normal embryonic fibroblasts with the E-cadherin gene converts them to the epithelial phenotype. It may be possible in the future to manipulate the tissue phenotype of diseased cells to the advantage of the animal.

Referenced Author (RAU)	Year (RPY)	VOL (RVL)	PG (RPG)	Referenced Work (RWK)	
HAY E D	1985	10	174	EXP BIOL MED	<--

L20 ANSWER 16 OF 26 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 92:703148 SCISEARCH
THE GENUINE ARTICLE: KA675
TITLE: DYNAMICS OF BETA(1) INTEGRIN-MEDIATED ADHESIVE CONTACTS IN MOTILE FIBROBLASTS
AUTHOR: REGEN C M (Reprint); HORWITZ A F
CORPORATE SOURCE: UNIV ILLINOIS, DEPT CELL & STRUCT BIOL, 505 S GOODWIN AVE, 506 MORRILL HALL, URBANA, IL, 61801 (Reprint)
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF CELL BIOLOGY, (DEC 1992) Vol. 119, No. 5, pp. 1347-1359.
ISSN: 0021-9525.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 40

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Motile chick skeletal fibroblasts adhere to a laminin substrate by means of clustered beta1 integrins. These integrin "macroaggregates" are similar to classic focal contacts but do not appear dark under

interference-reflection microscopy. They contain alpha5 integrin and are associated with extracellular fibronectin. To study their behavior during cell movement, time-lapse, low-light video microscopy was used to image integrins on living cells tagged with a fluorescent anti-beta1 integrin antibody.

Integrin macroaggregates remain fixed with respect to the substratum, despite the fact that they fluctuate in size, density, and shape over a period of minutes. Upon detachment of the cell rear, as much as 85% of the beta1 integrin density of a macroaggregate remains behind on the substrate, along with both alpha5 integrin and fibronectin. Release of the cell rear does not involve cleavage of the beta1 integrin cytoplasmic domain from the remainder of the protein. These results indicate that cell motility does not require regulated detachment of integrin receptors from the substrate. On the other hand, cytoskeletal components and a variable fraction of the integrins are carried forward with the cell during detachment, suggesting that some type of cortical disassembly process does occur.

Integrin macroaggregate structures are not recycled intact after detachment of the cell rear from the substrate. They do not persist on the cell surface, nor can they be seen to be engulfed by vesicles; yet, some of the individual integrins that make up these macroaggregates are eventually transported forward by both vesicular and cell-surface routes. Antibody-tagged integrins accumulate in dense patches at the lateral edges and dorsal surface of the cell, and move forward on the cell surface. The tagged integrins also enter cytoplasmic vesicles, which move forward within the cytoplasm.

Macroaggregates generally form and grow at the cell front; however, application of fluorescent antibody causes integrins to disappear from the leading edge. Therefore, it has not been possible to directly visualize the recycling of the forward moving tagged integrins into new macroaggregates at the cell front. Surprisingly, under these conditions cells move normally despite the absence of any delivery of tagged integrin to the leading edge, indicating that recycling of integrins to the lamella is not required for apparently normal motility.

Referenced Author (RAU)	Year (RPY)	VOL (RVL)	PG (RPG)	Referenced Work (RWK)	
HAY E D	1985	10	174	EXP BIOL MED	<--

L20 ANSWER 17 OF 26 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 91:205742 SCISEARCH
THE GENUINE ARTICLE: FE611
TITLE: ROLE OF CELL MATRIX CONTACTS IN CELL-MIGRATION AND EPITHELIAL-MESENCHYMAL TRANSFORMATION
AUTHOR: HAY E D (Reprint)
CORPORATE SOURCE: HARVARD UNIV, SCH MED, DEPT ANAT & CELLULAR BIOL, BOSTON, MA, 02115
COUNTRY OF AUTHOR: USA
SOURCE: CELL DIFFERENTIATION AND DEVELOPMENT, (1990) Vol. 32, No. 3, pp. 367-376.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 40

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Epithelial cells make contact with extracellular matrix via receptors on the basal surface that interact with the basal actin cortex. In 3D matrix, the mesenchymal cell makes contact with matrix all around its circumference via similar receptors. When moving, the fibroblast is constantly constructing a new front end. We postulate in a 'fixed cortex' theory of cell motility that the circumferential actin cortex is firmly attached to matrix and that the myosin-rich endoplasm slides past it into the continually forming new front end. During epithelial-mesenchymal

transformation, the presumptive mesenchymal cell seems to turn on the new front end mechanism as a way of emigrating from the epithelium into the underlying matrix with which it makes 'fixed' contacts. Master genes may exist that regulate the expression of epithelial genes on the one hand, and mesenchymal genes on the other.

Referenced Author (RAU)	Year (RPY)	VOL (RVL)	PG (RPG)	Referenced Work (RWK)	
HAY E D	1985	10	174	EXP BIOL MED	<--

L20 ANSWER 18 OF 26 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 90:369664 SCISEARCH
THE GENUINE ARTICLE: DM259
TITLE: EFFECTS OF SUBSTRATA ON THE POLARIZATION OF BOVINE ENDOMETRIAL EPITHELIAL-CELLS INVITRO
AUTHOR: MUNSON L (Reprint); WILKINSON J E; SCHLAFER D H
CORPORATE SOURCE: CORNELL UNIV, NEW YORK STATE COLL VET MED, DEPT PATHOL, ITHACA, NY, 14853; UNIV TENNESSEE, COLL VET MED, DEPT PATHOBIOL, KNOXVILLE, TN, 37996
COUNTRY OF AUTHOR: USA
SOURCE: CELL AND TISSUE RESEARCH, (1990) Vol. 261, No. 1, pp. 155-161.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 28

Referenced Author (RAU)	Year (RPY)	VOL (RVL)	PG (RPG)	Referenced Work (RWK)	
HAY E D	1985	10	174	EXP BIOL MED	<--

L20 ANSWER 19 OF 26 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 89:649617 SCISEARCH
THE GENUINE ARTICLE: CE571
TITLE: CELL-MIGRATION INTO NEURAL-TUBE LUMEN PROVIDES EVIDENCE FOR THE FIXED CORTEX THEORY OF CELL MOTILITY
AUTHOR: BILOZUR M E; HAY E D (Reprint)
CORPORATE SOURCE: HARVARD UNIV, SCH MED, DEPT ANAT & CELLULAR BIOL, 220 LONGWOOD AVE, BOSTON, MA, 02115
COUNTRY OF AUTHOR: USA
SOURCE: CELL MOTILITY AND THE CYTOSKELETON, (1989) Vol. 14, No. 4, pp. 469-484.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 68

Referenced Author (RAU)	Year (RPY)	VOL (RVL)	PG (RPG)	Referenced Work (RWK)	
HAY E D	1985	10	174	EXP BIOL MED	<--

L20 ANSWER 20 OF 26 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 89:649615 SCISEARCH
THE GENUINE ARTICLE: CE571
TITLE: THEORY FOR EPITHELIAL-MESENCHYMAL TRANSFORMATION BASED ON THE FIXED CORTEX CELL MOTILITY MODEL
AUTHOR: HAY E D (Reprint)
CORPORATE SOURCE: HARVARD UNIV, SCH MED, DEPT ANAT & CELLULAR BIOL, BOSTON, MA, 02115 (Reprint)
COUNTRY OF AUTHOR: USA
SOURCE: CELL MOTILITY AND THE CYTOSKELETON, (1989) Vol. 14, No. 4, pp. 455-457.
DOCUMENT TYPE: General Review; Journal

FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 13

Referenced Author (RAU)	Year (RPY)	VOL (RVL)	PG (RPG)	Referenced Work (RWK)	
HAY E D	1985	10	174	EXP BIOL MED	<--

L20 ANSWER 21 OF 26 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 89:577421 SCISEARCH
THE GENUINE ARTICLE: AY507
TITLE: TRANSMISSION ELECTRON-MICROSCOPY OF THE CYTOSKELETON OF MIGRATORY AND INVASIVE CELLS
AUTHOR: MCGARVEY T (Reprint); PERSKY B
CORPORATE SOURCE: LOYOLA UNIV, DEPT ANAT, MAYWOOD, IL, 60153 (Reprint)
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF ELECTRON MICROSCOPY TECHNIQUE, (1989) Vol. 13, No. 3, pp. 272-273.
DOCUMENT TYPE: Note; Journal
FILE SEGMENT: LIFE; ENGI
LANGUAGE: ENGLISH
REFERENCE COUNT: 5

Referenced Author (RAU)	Year (RPY)	VOL (RVL)	PG (RPG)	Referenced Work (RWK)	
HAY E D	1985	10	174	EXP BIOL MED	<--

L20 ANSWER 22 OF 26 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 89:541935 SCISEARCH
THE GENUINE ARTICLE: AV254
TITLE: DIFFERENTIATION OF MUSCULOSKELETAL TISSUES
AUTHOR: NATHANSON M A (Reprint)
CORPORATE SOURCE: UNIV MED & DENT NEW JERSEY, NEW JERSEY MED SCH, DEPT ANAT, NEWARK, NJ, 07103 (Reprint)
COUNTRY OF AUTHOR: USA
SOURCE: INTERNATIONAL REVIEW OF CYTOLOGY, (1989) Vol. 116, pp. 89-164.
DOCUMENT TYPE: General Review; Journal
LANGUAGE: ENGLISH
REFERENCE COUNT: 427

Referenced Author (RAU)	Year (RPY)	VOL (RVL)	PG (RPG)	Referenced Work (RWK)	
HAY E D	1985	10	174	EXP BIOL MED	<--

L20 ANSWER 23 OF 26 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 89:471823 SCISEARCH
THE GENUINE ARTICLE: AN877
TITLE: EXTRACELLULAR-MATRIX, CELL SKELETONS, AND EMBRYONIC-DEVELOPMENT
AUTHOR: HAY E D (Reprint)
CORPORATE SOURCE: HARVARD UNIV, SCH MED, DEPT ANAT & CELLULAR BIOL, 220 LONGWOOD AVE, BOSTON, MA, 02115 (Reprint)
COUNTRY OF AUTHOR: USA
SOURCE: AMERICAN JOURNAL OF MEDICAL GENETICS, (1989) Vol. 34, No. 1, pp. 14-29.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 63

Referenced Author (RAU)	Year (RPY)	VOL (RVL)	PG (RPG)	Referenced Work (RWK)

HAY E D	1985	10	174	EXP BIOL MED	<--

L20 ANSWER 24 OF 26 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 88:642349 SCISEARCH
THE GENUINE ARTICLE: Q9302
TITLE: SEA-URCHIN PRIMARY MESENCHYME CELLS - RELATION OF CELL POLARITY TO THE EPITHELIAL MESENCHYMAL TRANSFORMATION
AUTHOR: ANSTROM J A (Reprint); RAFF R A
CORPORATE SOURCE: WAKE FOREST UNIV, BOWMAN GRAY SCH MED, DEPT ANAT, WINSTON SALEM, NC, 27103 (Reprint); INDIANA UNIV, INST MOLEC & CELLULAR BIOL, BLOOMINGTON, IN, 47405; INDIANA UNIV, DEPT BIOL, BLOOMINGTON, IN, 47405
COUNTRY OF AUTHOR: USA
SOURCE: DEVELOPMENTAL BIOLOGY, (1988) Vol. 130, No. 1, pp. 57-66.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 20

Referenced Author (RAU)	Year (RPY)	VOL (RVL)	PG (RPG)	Referenced Work (RWK)	
HAY E D	1985	10	174	EXP BIOL MED	<--

L20 ANSWER 25 OF 26 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 87:588207 SCISEARCH
THE GENUINE ARTICLE: K4291
TITLE: PHYSICOCHEMICAL ASPECTS OF THE GIANT MULTINUCLEATE CELL-FORMATION
AUTHOR: SMETANA K (Reprint); SULC J; KRCOVA Z
CORPORATE SOURCE: CHARLES UNIV, FAC MED, DEPT ANAT, CS-12800 PRAGUE, CZECHOSLOVAKIA (Reprint); CZECHOSLOVAK ACAD SCI, INST MACROMOLEC CHEM, CS-11142 PRAGUE 1, CZECHOSLOVAKIA
COUNTRY OF AUTHOR: CZECHOSLOVAKIA
SOURCE: EXPERIMENTAL AND MOLECULAR PATHOLOGY, (1987) Vol. 47, No. 2, pp. 271-278.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 36

Referenced Author (RAU)	Year (RPY)	VOL (RVL)	PG (RPG)	Referenced Work (RWK)	
HAY E D	1985	10	174	EXP BIOL MED	<--

L20 ANSWER 26 OF 26 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 86:286591 SCISEARCH
THE GENUINE ARTICLE: C3196
TITLE: INTRACELLULAR-LOCALIZATION OF MESSENGER-RNAS FOR CYTOSKELETAL PROTEINS
AUTHOR: LAWRENCE J B (Reprint); SINGER R H
CORPORATE SOURCE: UNIV MASSACHUSETTS, DEPT ANAT, 55 LAKE AVE N, WORCESTER, MA, 01605 (Reprint)
COUNTRY OF AUTHOR: USA
SOURCE: CELL, (1986) Vol. 45, No. 3, pp. 407-415.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 51

Referenced Author (RAU)	Year (RPY)	VOL (RVL)	PG (RPG)	Referenced Work (RWK)	
HAY E D	1985	10	174	EXP BIOL MED	<--

=> d ibib ab

L29 ANSWER 1 OF 1 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN
ACCESSION NUMBER: 81:309348 SCISEARCH
THE GENUINE ARTICLE: LV187
TITLE: MECHANISM OF RETRACTION OF THE **TRAILING** EDGE DURING FIBROBLAST MOVEMENT
AUTHOR: **CHEN W T (Reprint)**
CORPORATE SOURCE: YALE UNIV, DEPT BIOL, NEW HAVEN, CT, 06520
COUNTRY OF AUTHOR: USA
SOURCE: **JOURNAL OF CELL BIOLOGY**, (1981) Vol. 90, No. 1, pp. 187-200.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 68

In the previous 26 entries, that cited the E D Hay article, this was the second most cited article (abstract was available)

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=> fil capl
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FILE COVERS 1907 - 29 Sep 2003  VOL 139 ISS 14
FILE LAST UPDATED: 28 Sep 2003  (20030928/ED)

  This file contains CAS Registry Numbers for easy and accurate
  substance identification.


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L32        3646 SEA FILE=CAPLUS ABB=ON  L30(3A)L31
L33      599322 SEA FILE=CAPLUS ABB=ON  MOVE? OR MOVING OR MIGRAT? OR LOCOMOT?
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L36       89819 SEA FILE=CAPLUS ABB=ON  CELL#(A)CULTUR?
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L47         255 SEA FILE=CAPLUS ABB=ON  L30 AND L46
L48          16 SEA FILE=CAPLUS ABB=ON  L47 AND 9/SC,SX - Section 103 Rejection
L49           2 SEA FILE=CAPLUS ABB=ON  A!!AYS/TI AND L48                  method


L30      464540 SEA FILE=CAPLUS ABB=ON  (TRACE# OR PATH# OR TRAIL#)
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L45       31912 SEA FILE=CAPLUS ABB=ON  MOTIL?
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L50           4 SEA FILE=CAPLUS ABB=ON  L48 AND SPERM?
L51           2 SEA FILE=CAPLUS ABB=ON  L50 AND VAP


=> s l38 or l44 or l49 or l51

L92           6 L38 OR L44 OR L49 OR L51

=> fil wpids

FILE 'WPIDS' ENTERED AT 11:29:34 ON 29 SEP 2003
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FILE LAST UPDATED:          28 SEP 2003      <20030928/UP>
MOST RECENT DERWENT UPDATE:     200362        <200362/DW>
DERWENT WORLD PATENTS INDEX SUBSCRIBER FILE, COVERS 1963 TO DATE


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>>> PATENT IMAGES AVAILABLE FOR PRINT AND DISPLAY <<<

>>> FOR A COPY OF THE DERWENT WORLD PATENTS INDEX STN USER GUIDE,
    PLEASE VISIT:
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    GUIDES, PLEASE VISIT:
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=> d que l61;d que l66; d que l70; d que l73; d que l76

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L57     1331323 SEA FILE=WPIDS ABB=ON  MOVE? OR MOVING OR MIGRAT? OR LOCOMOT?
                OR MOTIL? OR MOBILI? OR CHEMOTAX?
L58      398080 SEA FILE=WPIDS ABB=ON  CELL# OR CELLULAR?
L59        2146 SEA FILE=WPIDS ABB=ON  L58(3A)(L55 OR L56)
L60          45 SEA FILE=WPIDS ABB=ON  L59 (10A)L57
L61           6 SEA FILE=WPIDS ABB=ON  L60 AND (NERVE OR SELECTION OR ISOTOPES
                OR HARVESTING OR CORPUSCLE#)/TI



L55       35478 SEA FILE=WPIDS ABB=ON  TRACE#
L56      328813 SEA FILE=WPIDS ABB=ON  PATH# OR TRAIL#
L62        2802 SEA FILE=WPIDS ABB=ON  L56(1A)(L55 OR L56)
L63       83809 SEA FILE=WPIDS ABB=ON  J04/DC AND S/DC
L64          20 SEA FILE=WPIDS ABB=ON  L62 AND L63
L66           3 SEA FILE=WPIDS ABB=ON  L64 AND (BLOOD OR MIXER OR LABORATORY)/T
                I



L55       35478 SEA FILE=WPIDS ABB=ON  TRACE#
L56      328813 SEA FILE=WPIDS ABB=ON  PATH# OR TRAIL#
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L57     1331323 SEA FILE=WPIDS ABB=ON  MOVE? OR MOVING OR MIGRAT? OR LOCOMOT?
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L58      398080 SEA FILE=WPIDS ABB=ON  CELL# OR CELLULAR?
L63       83809 SEA FILE=WPIDS ABB=ON  J04/DC AND S/DC
L67         661 SEA FILE=WPIDS ABB=ON  (L55 OR L56)(1A)L58
L69           7 SEA FILE=WPIDS ABB=ON  L67 AND L63 AND L57
L70           3 SEA FILE=WPIDS ABB=ON  (CORPUSCLE OR CORNEAL OR SURFACE)/TI
                AND L69



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                OR MOTIL? OR MOBILI? OR CHEMOTAX?
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L73           2 SEA FILE=WPIDS ABB=ON  L71 AND (EXTRACT OR ALGAE)/TI



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                OR MOTIL? OR MOBILI? OR CHEMOTAX?
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L67         661 SEA FILE=WPIDS ABB=ON  (L55 OR L56)(1A)L58
L74      201656 SEA FILE=WPIDS ABB=ON  ANALY?
L76           1 SEA FILE=WPIDS ABB=ON  L67(5A)L74 AND L57


=> s l61 or l66 or l70 or l73 or l76

L93          12 L61 OR L66 OR L70 OR L73 OR L76

=> fil biosis

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=> d que l84; d que l87;d que l91

L77       91271 SEA FILE=BIOSIS ABB=ON  TRACE OR TRACES OR TRACK# OR PATH# OR
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L79      762482 SEA FILE=BIOSIS ABB=ON  MOVE? OR MOVING OR MIGRAT? OR LOCOMOT?
                OR MOTIL? OR MOBILI? OR CHEMOTAX?
L80         163 SEA FILE=BIOSIS ABB=ON  L78(3A)L77(15A)L79
L81      937016 SEA FILE=BIOSIS ABB=ON  CULTUR? OR CELL LINE#
L82          40 SEA FILE=BIOSIS ABB=ON  L80 AND L81
L83      108759 SEA FILE=BIOSIS ABB=ON  IMPLANT?
L84           1 SEA FILE=BIOSIS ABB=ON  L82 AND L83



L77       91271 SEA FILE=BIOSIS ABB=ON  TRACE OR TRACES OR TRACK# OR PATH# OR
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L77       91271 SEA FILE=BIOSIS ABB=ON  TRACE OR TRACES OR TRACK# OR PATH# OR
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L89     2020385 SEA FILE=BIOSIS ABB=ON  ANALY?
L90          16 SEA FILE=BIOSIS ABB=ON  L88 AND L89 AND L79 AND L81
L91           6 SEA FILE=BIOSIS ABB=ON  L90 AND (ADHESI? OR LAPSE OR FORCE OR
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=> s l84 or l87 or l91

L94         10 L84 OR L87 OR L91

=> dup rem l92,l94,l93
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PROCESSING COMPLETED FOR L94
PROCESSING COMPLETED FOR L93
L95         27 DUP REM L92 L94 L93 (1 DUPLICATE REMOVED)
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               ANSWERS '7-15' FROM FILE BIOSIS
               ANSWERS '16-27' FROM FILE WPIDS

=> d ibib ab 1-27; fil hom

L95  ANSWER 1 OF 27  CAPLUS  COPYRIGHT 2003 ACS on STN DUPLICATE 1
ACCESSION NUMBER:         2000:395617  CAPLUS
DOCUMENT NUMBER:          133:148089
TITLE:                    Cell adhesion and motility depend on nanoscale RGD
                          clustering
AUTHOR(S):                Maheshwari, Gargi; Brown, Gillian; Lauffenburger,
                          Douglas A.; Wells, Alan; Griffith, Linda G.
CORPORATE SOURCE:         Division of Bioengineering and Environmental Health,
                          Department of Chemical Engineering, and Center for
                          Biomedical Engineering, Massachusetts Institute of
                          Technology, Cambridge, MA, 02139, USA
SOURCE:                   Journal of Cell Science (2000), 113(10), 1677-1686
```

Searched by Barb O'Bryen, STIC 308-4291

CODEN: JNCSAI; ISSN: 0021-9533
PUBLISHER: Company of Biologists Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Integrin adhesion receptors play a crucial role in regulating interactions between cells and extracellular matrix (ECM). Integrin activation initiates multiple intracellular signaling pathways and results in regulation of cell functions such as motility, proliferation and differentiation. Two key observations regarding the biophys. nature of integrin-mediated cell-matrix interactions motivated the present study: (1) cell motility can be regulated by modulating the magnitude of cell-substratum adhesion, by varying cell integrin expression level, integrin-ECM binding affinity or substratum ECM surface d.; and (2) integrin clustering enables assembly of multiple cytoplasmic regulatory and structural proteins at sites of aggregated integrin cytoplasmic domains, activating certain intracellular signaling pathways. Here, using a minimal integrin adhesion ligand, YGRGD, we test the hypothesis that ligand clustering can affect cell migration in a manner related to its modulation of cell-substratum adhesion. We employ a synthetic polymer-linking method, which allows us to independently and systematically vary both the av. surface d. and the local (approx. 50 nm scale) spatial distribution of the YGRGD peptide, against a background otherwise inert with respect to cell adhesion. In this system, the ligand was presented in three alternative spatial distributions: singly, in clusters with an av. of five ligands per cluster, or in clusters with an av. of nine ligands per cluster; for each of these spatial distributions, a range of av. ligand densities (1,000-200,000 ligands/.mu.m2) were examd. Cluster spacing was adjusted in order to present equiv. av. ligand densities independently of cluster size. The murine NR6 fibroblast cell line was used as a model because its migration behavior on ECM in the presence and absence of growth factors has been well-characterized and it expresses integrins known to interact with the YGRGD peptide. Using time-lapse videomicroscopy and anal. of individual **cell** movement **paths**, we find that NR6 cells can migrate on substrate where adhesion is mediated solely by the YGRGD peptide. As previously obsd. for migration of NR6 cells on fibronectin, migration speed on YGRGD is a function of the av. surface ligand d. Strikingly, clustering of ligand significantly reduced the av. ligand d. required to support cell migration. In fact, non-clustered integrin ligands support cell attachment but neither full spreading nor haptokinetic or chemokinetic motility. In addn., by quantifying the strength of cell-substratum adhesion, we find that the variation of cell speed with spatial presentation of YGRGD is mediated via its effect on cell adhesion. These effects on motility and adhesion are also obsd. in the presence of epidermal growth factor (EGF), a known motility-regulating growth factor. Variation in YGRGD presentation also affects the organization of actin filaments within the cell, with a greater no. of cells exhibiting stress fibers at higher cluster sizes of YGRGD. Our observations demonstrate that cell motility may be regulated by varying ligand spatial presentation at the nanoscale level, and suggest that integrin clustering is required to support cell locomotion.

REFERENCE COUNT: 52 THERE ARE 52 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L95 ANSWER 2 OF 27 CAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 2002:854367 CAPLUS
DOCUMENT NUMBER: 139:113955

TITLE: Motility and fertilizing capacity of frozen/thawed common carp (Cyprinus carpio L.) **sperm** using dimethyl-acetamide as the main cryoprotectant

AUTHOR(S): Warnecke, Dietmar; Pluta, Hans-Jurgen
CORPORATE SOURCE: Institut fur Biologie, FG Okotoxikologie and Biochemie, Freie Universitat Berlin, Berlin, D-14195,

Germany
SOURCE: Aquaculture (2003), 215(1-4), 167-185
CODEN: AQCLAL; ISSN: 0044-8486
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB In the present study, motility and fertilizing capacity of fresh and their corresponding frozen/thawed common carp (Cyprinus carpio L.) **sperm** were investigated in order to evaluate several dimethyl-acetamide (DMA)-contg. cryo-diluents. **Sperm** motility was analyzed objectively through computer-assisted **sperm** motion anal. (CASA). In five consecutive test series, the effects on post-thaw motility of 11 different cryo-diluents added in a ratio of 1:6 were assessed. The cryo-diluent compn. varied by two different extenders, sugar addn. (sucrose or trehalose; 100-300 mM), and DMA concns. between 10% and 25%. Three freezing profiles were applied, which differed in freezing speed (averaged rate until -196 .degree.C: 3, 6, and 10 .degree.C min-1) and method (freezer-controlled vs. liq. nitrogen (LN2) vapor). For thawing, straws (0.25 mL) were immersed for 3 s in a water bath at 40 .degree.C. Optimal post-thaw motility results were obtained when using Cryo3 (modified Kurokura's extender 2 (MK2)/200 mM sucrose/15% DMA) and Cryo10 (MK2/200 mM trehalose/20% DMA) in combination with profile III (10 .degree.C min-1, above LN2). Maximum initial (15-20 s post-activation) motility rates exhibited 40.+-.6% and reached about half the values of the corresponding fresh **sperm**. Initial averaged **path** velocity (**VAP**) was reduced from 70-90 .mu.m s-1 with fresh **sperm** to max. values of 60-65 .mu.m s-1 with frozen/thawed **sperm**. In a final test series, the fertilizing capacity of **sperm** frozen with Cryo3 and Cryo10 was investigated. Calcd. from the total no. of eggs, hatching rate was 80.+-.2% and percentage of swim-up-stage larvae was 78.+-.2% when **sperm** were frozen for 6 days in Cryo10. These results were nearly identical with the control values (fresh **sperm**). **Sperm** frozen with Cryo3 and stored for 349 days in LN2 still produced 38.+-.5% of healthy larvae. Five minutes equilibration time of fresh **sperm** with Cryo10 resulted in a steep decrease of motile cells to levels of frozen/thawed **sperm**, but 68.+-.7% of the embryos developed normally. Using DMA as internal cryoprotectant in combination with the MK2/sugar extender proved to be very suitable for cryopreservation of common carp **sperm**, esp. with regard to fertilization and hatching success.
REFERENCE COUNT: 40 THERE ARE 40 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L95 ANSWER 3 OF 27 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2001:803605 CAPLUS
DOCUMENT NUMBER: 136:67572
TITLE: Effects of differing gentamicin concentrations in semen extender on **spermatozoa** motility and velocity in fresh and cooled stallion semen
AUTHOR(S): Bennett-Wimbush, K.; Andrews, A.; Jones, J.; Musolf, B.
CORPORATE SOURCE: Ohio State University Agricultural Technical Institute, Wooster, OH, 44691, USA
SOURCE: Journal of Applied Animal Research (2001), 20(1), 41-47
CODEN: JANREH; ISSN: 0971-2119
PUBLISHER: Garuda Scientific Publications
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Ten light-horse stallions (ages 5-16) were used to det. the effects of differing concns. of gentamicin sulfate in semen extender on **sperm** motility and velocity in both fresh and cooled stallion semen. Semen was collected once from all stallions from Oct. 1997 through Mar. 1998.

Immediately after collection, semen was extended with a dried-skim milk based extender (pH = 7.0) contg. either 0, 500 or 2000 .mu.g/mL gentamicin sulfate to a final concn. of 10 .times. 106 **spermatozoa**/mL. Av. **path** velocity decreased (p<.01) in cooled semen (44.2.+-.2.3 .mu.m/s) when compared with fresh semen (65.1.+-.2.2 .mu.m/s). However, there was no difference in av. **path** velocity (**VAP**) between samples extended with either 0, 500 or 2000 .mu.g/mL of gentamicin sulfate in either fresh or cooled semen. Per cent motility significantly decreased (p<.01) in cooled semen. In addn., high concns. of gentamicin sulfate (2000 .mu.g/mL) decreased (p<.01) percent motility in fresh semen. This effect was more pronounced in cooled semen in presence of 500 or 2000 .mu.g/mL of gentamicin sulfate. It is concluded that high concns. of gentamicin sulfate appear to be detrimental to **sperm** motility, but not velocity.

REFERENCE COUNT: 9 THERE ARE 9 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L95 ANSWER 4 OF 27 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1999:614248 CAPLUS
DOCUMENT NUMBER: 131:225804
TITLE: Systems having vascularized perfused microtissue/micro-organ **arrays** and sensors
INVENTOR(S): Griffith, Linda G.; Tannenbaum, Steven R.; Powers, Mark J.; Domansky, Karel; Thompson, Charles D.
PATENT ASSIGNEE(S): Massachusetts Institute of Technology, USA
SOURCE: PCT Int. Appl., 102 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9947922	A2	19990923	WO 1999-US5974	19990318
W: CA, JP				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				

PRIORITY APPLN. INFO.: US 1998-78452P P 19980318

AB Systems including (1) a micromatrix and perfusion assembly suitable for seeding and attachment of cells within the matrix and for morphogenesis of seeded cells into complex, hierarchical tissue or organ structures, wherein the matrix includes channels or vessels through which culture medium, oxygen, or other nutrient or body fluids can be perfused while controlling gradients of nutrients and exogenous metabolites throughout the perfusion **path** independently of perfusion rate, and (2) sensor means for detecting changes in either cells within the matrix or in materials exposed to the cells, have been developed. Methods for making the micromatrices include micromachining, micromolding, embossing, laser drilling, and electro deposition machining. Cells can be of one or more types, either differentiated or undifferentiated. In a preferred embodiment, the matrix is seeded with a mixt. of cells including endothelial cells which will line the channels to form "blood vessels", and at least one type of parenchymal cells, such as hepatocytes, pancreatic cells, or other organ cells. The system can be used to screen materials for an effect on the cells, for an effect of the cells on the materials (for example, in a manner equiv. to tissue metab. of a drug), or to test a material on a biol. that must first infect cells or tissues, such as viruses. The app. also can be used to provide a physiol. environment for expansion of stem cells, or for enabling gene therapy in vitro.

L95 ANSWER 5 OF 27 CAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 1998:567905 CAPLUS
DOCUMENT NUMBER: 129:341319
TITLE: **Assays** of leukocyte locomotion and chemotaxis
AUTHOR(S): Wilkinson, P. C.
CORPORATE SOURCE: Dep. Immunol., Univ. Glasgow (Western Infirmary), Glasgow, G11 6NT, UK
SOURCE: Journal of Immunological Methods (1998), 216(1-2), 139-153
CODEN: JIMMBG; ISSN: 0022-1759
PUBLISHER: Elsevier Science B.V.
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review with 76 refs. This review discusses the range of methods which are currently available for measuring locomotion and chemotaxis of leukocytes in vitro, their history, and some definitions of terms. Assays of the net migration of large cell populations, such as the filter assay are the most popular and are useful for identifying chemoattractant mols., but give no direct information about how these mols. influence the speed and direction of cell movement (chemokinesis and chemotaxis). Visual assays including measures of orientation in gradients and time-lapse filming give detailed information about cell **paths** and direct evidence for chemotaxis and chemokinesis. The polarization assay is a useful visual screening assay. Assays which simulate the situation in living tissues are becoming more popular and include migration through collagen or fibrin gels or through monolayers of vascular endothelium. Locomotion is a complex process, no single assay gives full information and the use of more than one assay is recommended.
REFERENCE COUNT: 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L95 ANSWER 6 OF 27 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 1997:634596 CAPLUS
DOCUMENT NUMBER: 127:304864
TITLE: Fast Blue, a fluorescent tracer in glioma cell culture, affects cell proliferation and motility
AUTHOR(S): Vince, Giles Hamilton; Bouterfa, Hakim; Goldbrunner, Roland; Roosen, Klaus; Tonn, Jorg Christian
CORPORATE SOURCE: Dep. Neurosurgery, Univ. Wurzburg, Wurzburg, 97080, Germany
SOURCE: Neuroscience Letters (1997), 233(2,3), 148-150
CODEN: NELED5; ISSN: 0304-3940
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The azo-dye, Fast Blue (FB), initially employed for retrograde neuronal tracing is increasingly used in cell invasion and migration studies to detect living cells in monolayer and glioma tumor cell spheroid models. As yet, it is assumed that a cell stained with a tracker dye retains the characteristics of the original cell. The following expts. compared the adhesion, migration and proliferation properties of the cell lines U373 and GaMG with and without FB staining. FB staining reduced cell adhesion and proliferative activity and also had a significant inhibitory effect on cell migration. From the results presented it follows that FB staining markedly influences basic cell characteristics.

L95 ANSWER 7 OF 27 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2002:151822 BIOSIS
DOCUMENT NUMBER: PREV200200151822
TITLE: Murine Sca-1+/Lin- cells and the human AML **cell line** KG1a, exhibit multiple **pseudopod** morphologies and non-Markovian **movement** during **locomotion**.

AUTHOR(S): Francis, Karl (1); Gemmen, Greg (1); Palsson, Bernhard (1); Carrier, Ewa
CORPORATE SOURCE: (1) Bioengineering, UCSD, La Jolla, CA USA
SOURCE: Blood, (November 16, 2001) Vol. 98, No. 11 Part 2, pp. 115b. http://www.bloodjournal.org/. print.
Meeting Info.: 43rd Annual Meeting of the American Society of Hematology, Part 2 Orlando, Florida, USA December 07-11, 2001
ISSN: 0006-4971.
DOCUMENT TYPE: Conference
LANGUAGE: English

AB The **chemotaxis** of immature hematopoietic cells (IHCs) has been studied mostly via population (trans-membrane) assays while the actual mechanisms of cell **locomotion** have been defined by individual tracking of mature cell types. In this study, murine Sca-1+/Lin- IHCs and the human AML5 **cell line**, KG1a, were tracked using time-lapse microscopy. These cells were observed to exhibit a variety of mechanisms/morphologies during **migration** which include the classic "hand mirror" shape, broad, flat lamellipodia, trailing uropodia, dynamic filopodia, and retraction fibers. Time lapse observations of trans-membrane assays revealed long, thin magnupodia passing through the pores, while other measurements show magnupods can generate forces capable of accelerating a cell to a velocity of 6.5 microns/min. Many of these mechanisms have been reported separately for differentiated cells however, it is of interest that they are all present in immature cells. Furthermore, a widely used model for describing cellular **locomotion** assumes that **cell tracks** can be described by a random walk process. We applied two **analysis** methods to Sca-1+/Lin- **cell tracks** collected in our laboratory to examine the validity of this assumption. The first method assumed a Markovian persistent random walk process to compute cell speeds and persistence times. The second method computed speed and turn angle distributions directly from the cell displacements. Additionally, the velocity autocorrelation function, the exponential decay of which is the fundamental tenet of the persistent random walk model, was shown to be non-Markovian for IHCs. To test whether speed and turn angle distributions similar to those observed for IHCs could produce non-Markovian behaviour, we generated simulated **cell tracks**. Varying the distribution characteristics produced tracks with both Markovian and non-Markovian correlations. These observations of non-Markovian behaviour bring into question the applicability of the persistent random walk model to IHC tracks. Finally, the effects of the growth factor GM-CSF on murine IHC tracks were measured using speed and turn angle distributions. The presence of GM-CSF induces highly signifigant changes in the speed and turn angle distributions, indicating an increase in **motility**. These data provide further insight into the mechanisms of hematopoietic stem cell **motility**, **mobilization** and homing as well as the methods used to **analyze** IHC **migration** data.

L95 ANSWER 8 OF 27 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1999:490383 BIOSIS
DOCUMENT NUMBER: PREV199900490383
TITLE: Topography of **cell traces** studied by atomic **force** microscopy.
AUTHOR(S): Zimmermann, Heiko; Hagedorn, Rolf; Richter, Ekkehard; Fuhr, Guenter (1)
CORPORATE SOURCE: (1) Lehrstuhl fuer Membranphysiologie, Institut fuer Biologie, Mathematisch-Naturwissenschaftliche Facultaet I, Humboldt-Universitaet zu Berlin, Invalidenstrasse 42, D-10115, Berlin Germany
SOURCE: European Biophysics Journal, (1999) Vol. 28, No. 6, pp. 516-525.
ISSN: 0175-7571.

DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB **Migrating** adherent cells release material onto artificial substrates like glass and silicon while **moving**. Traces of mouse fibroblasts (L929) have been visualised by atomic force microscopy (AFM). "Non-contact" mode AFM in a liquid environment can extract topographic information from these traces. This dynamic mode allows the study of these soft structures without damage or compression. The AFM images show crossing and branching networks (with specific angles of branching), structured patches, nodular elements, linear elements with irregular height and other features. Fourier **analysis** of segment spacing in the strands is presented. These spatial features of fibroblast traces are strong indications that actin linked to structural proteins is involved in the formation of **cell traces**. We also give methods for trace preparation and undistorted imaging and discuss further perspectives.

L95 ANSWER 9 OF 27 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1998:507473 BIOSIS
DOCUMENT NUMBER: PREV199800507473
TITLE: **Cell traces**: Footprints of individual **cells** during **locomotion** and **adhesion**.
AUTHOR(S): Fuhr, Guenter (1); Richter, Ekkehard; Zimmermann, Heiko; Hitzler, Hermine; Niehus, Horst; Hagedorn, Rolf
CORPORATE SOURCE: (1) Humboldt-Univ. Berlin, Inst. Biol., Invalidenstr. 42, D-10115 Berlin Germany
SOURCE: Biological Chemistry, (Aug.-Sept., 1998) Vol. 379, No. 8-9, pp. 1161-1173.
ISSN: 1431-6730.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Animal **cells** release **traces** of material onto glass or silicon **surface*** during adhesion and **migration**. This little studied phenomenon is a widespread and normal concomitant of cell migration. The paper introduces the study of such material. The traces can be visualised by different microscopic techniques (e.g. TIRF, IRM, CLSM, AFM, SEM). Cell traces typical for different **cell lines** (NIH 3T3 and L929 mouse fibroblasts, mouse macrophages, mouse sarcoma cells and human osteosarcoma cells) are shown and discussed. There are well organised structures such as different linear and nodular elements as well as patches. Traces can extend up to some hundred micrometers from the cell, but the dimensions of the linear elements are in the submicron range. Cell traces are not identical with focal contacts but can include them. A first classification of basic elements is proposed. It allows an estimation of the total volume and **surface** in comparison to the donor cell. Higher order structures are discussed and a first insight into the protein composition of traces produced by mouse fibroblasts is given. Our observations, together with the cell adhesion literature suggest that the amount of released material, its extent and chemical and structural properties depend on cell type and physiology as well as other external influences. Cell traces in combination with the adhesion pattern of the donor cell should give information about the activity and physiological status of individual cells, the mechanisms of cell **locomotion** and the molecular composition of the donor **cell** membrane. The **traces** might possibly be used as submicron elements for passive electric characterisation and biotechnological applications.

L95 ANSWER 10 OF 27 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1998:35649 BIOSIS
DOCUMENT NUMBER: PREV199800035649
TITLE: Contact of **sarcoma** cells with aligned fibroblasts

accelerates their displacement: Computer-assisted analysis of tumour cell locomotion in co-**culture**.
AUTHOR(S): Korohoda, Wlodzimmierz (1); Madeja, Zbigniew
CORPORATE SOURCE: (1) Dep. Cell Biology, J. Zurzycki Inst. Molecular Biology, Jagiellonian Univ., Mickiewicza 3, 31-120 Krakow Poland
SOURCE: Biochemistry and Cell Biology, (1997) Vol. 75, No. 3, pp. 263-276.
ISSN: 0829-8211.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English; French
AB The shape and locomotion of rat sarcoma XC cells on glass, polystyrene, and confluent monolayer **cultures** of aligned human skin fibroblasts were studied with quantitative, computer-assisted methods. The cell shape depended upon the substratum; the sarcoma cells seeded on fibroblasts assumed polarized shapes. The tumour cells emigrating from aggregates and in sparse **cultures** showed random locomotion when plated on glass or on the polystyrene **surface** of tissue **culture** dishes in isotropic conditions. However, when sarcoma cell aggregates were plated onto underlying aligned fibroblasts, the sarcoma cells showed contact guidance, migrating along the long axes of fibroblasts. Simultaneously, suppression of migration normal to the axis of fibroblasts orientation was observed. The sarcoma cells displaced a few times faster on aligned fibroblasts than under isotropic conditions in control **cultures**. This fast displacement was found to result from the less frequent cell turnings and straightening of cell trajectories (i.e., from klinokinesis), and not from an acceleration of cell **movement** and the longer **cell tracks** (i.e., not from orthokinesis). The presented results support the suggestion of Abercrombie (M. Abercrombie, 1979, Nature (London), 281: 259-262.) that tumour cells may be guided by the underlying normal cells when invading surrounding tissues and forming metastases.

L95 ANSWER 11 OF 27 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1997:15621 BIOSIS
DOCUMENT NUMBER: PREV199799314824
TITLE: Synergistic and hierarchical **adhesive** and topographic guidance of BHK cells.
AUTHOR(S): Britland, Stephen (1); Morgan, Hywel; Wojiak-Stodart, Beata; Riehle, Mathis; Curtis, Adam; Wilkinson, Chris
CORPORATE SOURCE: (1) Cent. Cell Engl., IBLS, Univ. Glasgow, Glasgow G12 8QQ UK
SOURCE: Experimental Cell Research, (1996) Vol. 228, No. 2, pp. 313-325.
ISSN: 0014-4827.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Guided cell **movement** is a fundamental process in development and regeneration. We have used microengineered **culture** substrates to study the interaction between model topographic and adhesive guidance cues in steering BHK cell orientation. Grooves 0.1, 0.5, 1.0, 3.0, and 6.0 mu-m deep together with pitch-matched aminosilane tracks 5, 12, 25, 50, and 100 mu-m wide were fabricated on fused silica substrates using photolithographic and dry-etching techniques. The cues were presented to the cells individually, simultaneously in parallel and orthogonally opposed. Cells aligned most strongly to 25-mu-m-wide adhesive tracks and to 5-mu-m-wide, 6-mu-m-deep grooves. Stress fibers and vinculin were found to align with the adhesive tracks and to the grooves and ridges. Cell alignment was profoundly enhanced on all surfaces that presented both cues in parallel. Cells were able to switch alignment from ridges to grooves, and vice versa, depending on the location of superimposed adhesive **tracks**. **Cells** aligned preferentially to adhesive tracks superimposed orthogonally over grooves of matched pitch, traversing

numerous grooves and ridges. The strength of the cues was more closely matched on narrower 3- and 6-mu-m-deep gratings with cells showing evidence of alignment to both cues. Confocal fluorescence microscopy revealed two groups of mutually opposed f-actin stress fibers within the same cell, one oriented with the topographic cues and the other with the adhesive cues. However, the adhesive response was consistently dominant. We conclude that cells are able to detect and respond to multiple guidance cues simultaneously. The adhesive and topographic guidance cues modeled here were capable of interacting both synergistically and hierarchically to guide cell orientation.

L95 ANSWER 12 OF 27 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1996:479541 BIOSIS
DOCUMENT NUMBER: PREV199699194797
TITLE: Control of cell **adhesion**, migration, and orientation on photochemically microprocessed **surfaces**.
AUTHOR(S): Matsuda, Takehisa (1); Sugawara, Takashi
CORPORATE SOURCE: (1) Dep. Bioengineering, National Cardiovascular Center Research Inst., 5-7-1 Fujishirodai, Suita, Osaka 565 Japan
SOURCE: Journal of Biomedical Materials Research, (1996) Vol. 32, No. 2, pp. 165-173.
ISSN: 0021-9304.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Using **surface**-photochemistry-driven microprocessing, striped patterns of cell-adhesive and nonadhesive domains were prepared on tissue-**culture** dishes. The width of striped patterns ranged from 20 to 130 mu-m. When endothelial cells were **cultured** on such dimensionally well-defined **surfaces**, cells adhered, **migrated**, and proliferated only on cell-adhesive domains. **Migration** potentials such as **tracks** of **moving cells** and **migration** rates were determined using a time-lapse video recording apparatus under a phase-contrast microscope and a computer-assisted image analyzer. The migration track in the direction of the width of the stripe-pattern was limited to the size of the width, and effective migratory distance over 400 min of observation was considerably reduced, to almost half that for a nontreated **surface**, whereas migratory rate was not changed by **surface** processing, irrespective of the stripe-pattern width. After a 2-day **culture**, oriented patterned cellular sheets were obtained. Cells were elongated and aligned along the axis of the striped pattern. The degrees of orientation and elongation were enhanced with a decrease of the line width. At the narrowest **surface** domain, cells only migrated back and forth, and eventually they became highly elongated and oriented along the axis of the domain. These results indicated that the adhesion area, migrating direction, and orientation of cells can be controlled by this method with micron-order precision. This method provides quantitative information on the kinetics of the migration process and the morphogenesis of the microprocessed **surface**.

L95 ANSWER 13 OF 27 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1995:120491 BIOSIS
DOCUMENT NUMBER: PREV199598134791
TITLE: Heterogeneity in **migration** of smooth muscle cells from normal and injured rat thoracic **aorta** in primary **culture**.
AUTHOR(S): Peronneau, Isabelle (1); Gavoille, Andre; Peronneau, Pierre; Dubray, Claude; Capron, Loic
CORPORATE SOURCE: (1) Unite INSERM 256, Hop. Broussais, 75674 Paris Cedex France
SOURCE: Cardiovascular Research, (1995) Vol. 29, No. 1, pp. 38-43.
ISSN: 0008-6363.

DOCUMENT TYPE: Article
LANGUAGE: English
AB Objective: Aside from proliferation, **migration** of smooth muscle cells is an essential component of the arterial sclerotic reaction. The aim of this study was to define a model to study **migration**. Methods: Primary **cultures** of smooth muscle cells were derived from normal or injured rat thoracic aorta. An image **analysis** system was used to **track cells migrating** out of the explants and measure the displacement of their centre of gravity. Results: **Migration** speeds for smooth muscle cells randomly sampled from the normal whole media were very heterogeneous. The media were therefore separated into three vertical segments. Cells from the middle third **migrated** faster than those from the upper and lower thirds, regardless of whether they originated from the anterior and posterior parts of the segment (P=0.001). Heparin (10 mu-g cntdot ml-1) only inhibited smooth muscle cell **migration** from the middle segment (P lt 0.001). **Migration** of smooth muscle cells from explants of aorta 3 and 14 d after injury was also studied using a balloon catheter. Three days after injury, cell velocity varied widely among the segments of the same media. In contrast, 14 d after injury cells from neointimal explants **migrated** homogeneously and at a slower rate than those obtained from normal media. Conclusions: These experiments show **migratory** variations among smooth muscle cells depending upon their position in the normal aorta and their state of activation after arterial injury. This variability must be taken into account when planning experiments to study smooth muscle cell **migration**.

L95 ANSWER 14 OF 27 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1994:30322 BIOSIS
DOCUMENT NUMBER: PREV199497043322
TITLE: Immunohistochemical investigation of tracks left by the migration of fibroblasts on titanium surfaces.
AUTHOR(S): Abiko, Y.; Brunette, D. M. (1)
CORPORATE SOURCE: (1) Dep. Oral Biol., Univ. British Columbia, 2199 Wesbrook Mall, Vancouver, BC V6T 1Z3 Canada
SOURCE: Cells and Materials, (1993) Vol. 3, No. 2, pp. 161-170. ISSN: 1051-6794.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Fibronectin, a major attachment protein, has been thought to be involved in pathway guidance, a process in which cells migrate along specific pathways within a tissue during development. Fibroblasts exhibit the phenomenon of contact guidance, the tendency of cells to be guided in their direction of migration by the shape of the substratum. The purpose of our study was to test the hypothesis that fibronectin tracks are deposited by fibroblasts moving on smooth and grooved titanium surfaces. The study was carried out on human gingival fibroblasts which were plated onto both smooth and grooved titanium substrata using medium containing either serum or fibronectin-depleted serum. The **migratory paths** of the **cells** were determined by time-lapse photography using reflected-light differential-interference-contrast optics. Anti-fibronectin antibody, 1 nm gold particle conjugated secondary antibody, and silver enhancement techniques were applied to the **cultured** cells, and the specimens observed in a scanning electron microscope using backscattered detection. By correlating the paths of the cells with the location of the fibronectin-containing material, it could be demonstrated that cells left behind fibronectin tracks on both smooth and grooved titanium surfaces. Fibronectin tracks appeared to be deposited more abundantly by fibroblasts **cultured** in medium with 5% serum depleted in fibronectin than in complete, i.e., non-depleted, 5% serum. On the grooved titanium substratum, the tracks were found on the ridges as well as on the floors and walls of the grooves. The fibronectin tracks are aligned with the grooves so that they would be expected to reinforce the

contact guidance produced by the substratum.

L95 ANSWER 15 OF 27 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1988:287838 BIOSIS
DOCUMENT NUMBER: BA86:16105
TITLE: A METHOD OF QUANTITATIVE **ANALYSIS** OF CELL **MIGRATION** USING A COMPUTERIZED TIME-**LAPSE** VIDEOMICROSCOPY.
AUTHOR(S): ZAMA N; KATOW H
CORPORATE SOURCE: LAB. BIOL., RIKKYO UNIV., NISHI-IKEBUKURO, TOKYO 171, JPN.
SOURCE: ZOOL SCI (TOKYO), (1988) 5 (1), 53-60.
CODEN: ZOSCEX. ISSN: 0289-0003.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB A quantitative **analysis** of the cell **migration** in vitro has been realized by examining a distance of the **migration** performed by the cells, and the results are represented by a format called the "cell **migration** pattern". We have composed a system which is capable to calculate the cell **migration** patterns, and to carry on such statistical tests as the parametrics as well as the non-parametrics to compare and evaluate the significant differences among the cell **migration** patterns. The system consists of an inverted phase contrast microscope, a video camera which is connected with the microscope, a monitor TV, a time-lapse video cassette recorder, a position **analyzer**, and a microcomputer. This system can **trace** 7 **cells** at a time and calculate above **migration** criterion. The system was applied to **analyze** the **migratory** behavior of the primary mesenchyme cells of the sea urchin blastulae in different **culture** conditions as a model case. Its desired functions were fully demonstrated in the present study.

L95 ANSWER 16 OF 27 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN
ACCESSION NUMBER: 2003-513567 [48] WPIDS
DOC. NO. NON-CPI: N2003-407646
DOC. NO. CPI: C2003-137500
TITLE: Cell guidance method, useful for controlling cell motility and/or growth, for peripheral **nerve** repair, for spinal trauma, or for treatment of paralysis, comprises defining a cell guidance path with a light source.
DERWENT CLASS: B04 D16 P81
INVENTOR(S): BETZ, T; EHRLICHER, A; KAS, J; MILNER, V; RAIZEN, M; KAES, J
PATENT ASSIGNEE(S): (BETZ-I) BETZ T; (EHRL-I) EHRLICHER A; (KASJ-I) KAS J; (MILN-I) MILNER V; (RAIZ-I) RAIZEN M; (TEXA) UNIV TEXAS SYSTEM
COUNTRY COUNT: 100
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2003042723	A2	20030522	(200348)*	EN	21
RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SK SL SZ TR TZ UG ZM ZW					
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VN YU ZA ZM ZW					
US 2003109040	A1	20030612	(200348)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2003042723 A2		WO 2002-US36456	20021113
US 2003109040 A1	Provisional	US 2001-333283P	20011114
		US 2002-293142	20021113

PRIORITY APPLN. INFO: US 2001-333283P 20011114; US 2002-293142 20021113

AB WO2003042723 A UPAB: 20030729

NOVELTY - A cell guidance method (M), comprising defining a cell guidance path with a light source, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) an apparatus (I), comprising:

(a) a stage to position a target cell; and

(b) a microscope to receive a light beam from a light source;

(2) an apparatus (II), comprising:

(a) a light source (120) to generate a light beam (130); and

(b) a unit for biasing a direction of lamellipodia growth of a cell based on a location of the light beam; and

(3) a system (III), comprising:

(a) a cell substrate for attachment of a cell;

(b) a light source to generate a light beam; and

(c) a device to control motility of the cell based on movement of the light beam to different points of different planes of the cell substrate.

ACTIVITY - Neuroprotective; Ophthalmological.

No biological data given.

MECHANISM OF ACTION - None given.

USE - (M) is useful in a cell controlling method, which comprises:

(a) illuminating a portion of a cell with a light source;

(b) defining a growth path for the portion of the cell; and

(c) controlling growth of a growth cone in a desired direction, where the portion of the cell has an accelerated growth in the desired direction.

The cell is a neuron, and the portion of the cell is a neural growth cone.

(I), (II) and (III) are useful for carrying out (M) (all claimed).

(M) is useful for guiding cells to produce a predetermined cellular structure, and/or a more elaborate cellular structure comprised of multiple cells, such as a network, for controlling cell motility/growth direction and/or growth rate of cells, particularly neurons, for in vitro circuits of real neurons, for peripheral nerve repair, for spinal trauma, to create in vitro neurons, as well as guiding nerves in vivo to form new connections or reattaching nerves in trauma cases, to direct neurons onto a biological/semiconductor interface, in synthetic prosthesis-sensory control, treatment for paralysis, biocomputing, replacing nerve guide channels, or reversing some cases of blindness by reattaching retinas. (M) contributes a large set of potential directions to the study of motility of growth cones and neural networks. (M) can connect neurons through synapses, wiring different axons and cells together to create new network nodes in a highly controlled fashion.

ADVANTAGE - (M) is dynamic, exceptionally accurate and can target a particular section of a growth cone with micron level precision. (M) is effective in three dimensions, moving easily between different points in the horizontal plane with mirrors or a movable stage. The three-dimensional capabilities of (M) makes it a natural choice for in vivo applications. (M) is a far less invasive than other methods. By interacting with the neuron only using light, (M) avoids contaminating the neurons or damaging them or surrounding tissue, with very precise control of the applied force. (M) can be implemented entirely with commercially available equipment, cutting out the monetary and time expense of custom components.

DESCRIPTION OF DRAWING(S) - The figure shows an exemplary apparatus for optical cell guidance.
Light source 120
Light beam 130
Dwg.1/3

L95 ANSWER 17 OF 27 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN
ACCESSION NUMBER: 2003-460902 [44] WPIDS
DOC. NO. NON-CPI: N2003-366595
DOC. NO. CPI: C2003-123076
TITLE: Micro **mixer**, used in chemical-analysis apparatus, has transduction flow **path**, interflow **path** and draining flow path which are formed by etching upper face of cell substrate.
DERWENT CLASS: B04 J02 **J04 S03**
PATENT ASSIGNEE(S): (FJIE) FUJI ELECTRIC CO LTD
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
JP 2002346355 A		20021203	(200344)*		5

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
JP 2002346355 A		JP 2001-158051	20010528

PRIORITY APPLN. INFO: JP 2001-158051 20010528
AB JP2002346355 A UPAB: 20030710
NOVELTY - The upper face of a cell substrate (11) is etched, to form a transduction flow path (13), an interflow path (14) and a draining flow path (15). The interflow path which has depth greater than the flow paths (13,15), has several convex-shaped portions (14a).
USE - Used in chemical-analysis apparatus and medical speciality apparatus.
ADVANTAGE - Enables effective mixing of trace amount of liquid, using a small and simple structure.
DESCRIPTION OF DRAWING(S) - The figure shows the top view and sectional view of the cell substrate in the micro mixer, as above. (Drawing includes non-English language text).
Cell substrate 11
Transduction flow path 13
Interflow path 14
Convex-shaped portions 14a
Draining flow path 15
Dwg.1/5

L95 ANSWER 18 OF 27 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN
ACCESSION NUMBER: 2000-431667 [37] WPIDS
DOC. NO. NON-CPI: N2000-322101
DOC. NO. CPI: C2000-131325
TITLE: Investigating cells from tracks they leave on a **surface**, useful for diagnosis and toxicological testing, also method for modifying **surface** properties of implant materials.
DERWENT CLASS: B04 D16 **J04 S03**
INVENTOR(S): FUHR, G; HAGEDORN, R; RICHTER, E; SHIRLEY, S G
PATENT ASSIGNEE(S): (EVOT-N) EVOTEC BIOSYSTEMS AG
COUNTRY COUNT: 27
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2000036415	A1	20000622	(200037)*	GE	34
RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE					
W: JP US					
DE 19857692	C1	20000824	(200041)		
EP 1144999	A1	20011017	(200169)	GE	
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI					

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2000036415	A1	WO 1999-EP9781	19991210
DE 19857692	C1	DE 1998-19857692	19981214
EP 1144999	A1	EP 1999-964544	19991210
		WO 1999-EP9781	19991210

FILING DETAILS:

PATENT NO	KIND		PATENT NO
EP 1144999	A1	Based on	WO 2000036415

PRIORITY APPLN. INFO: DE 1998-19857692 19981214

AB WO 200036415 A UPAB: 20000807

NOVELTY - Method for **cell trace**-based investigation of cells is new and comprises:

(i) applying the cells to an at least partly structured and/or surface-modified substrate; and

(ii) allowing them to **move** adherently over tracks on the surface so that they leave traces consisting of material residues (A) separated from the **cells**. These **traces** are then **analyzed**.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a device for performing this process;

(2) a method for **cell trace**-based culture of cells; and

(3) use of (A), formed on a substrate, for investigating properties of cells and for biocompatible modification of implant materials.

USE - Residues (A) are used:

(i) for examining properties of cells for medical (e.g. diagnostic or toxicological), biochemical and/or pharmacological purposes;

(ii) for biocompatible modification of the surface of implant materials; and

(iii) for culturing cells (specifically tissue-generating cells such as chondrocytes, osteoblasts or epithelial cells) or more generally to manipulate the interaction of cells with solid substrates.

ADVANTAGE - The method allows many different analytical techniques (high-specificity detection methods and destructive methods) to be combined, and many individual cells can be examined in parallel. By **analyzing traces**, the **cells** themselves remain intact and are still available for other uses. Implant materials coated with (A) are rendered biocompatible and have a significantly broadened spectrum of use.

DESCRIPTION OF DRAWING(S) - The diagram illustrates the device.

Region of surface to which cell adhesion is poor 12

Region of surface to which cell adhesion is good, defining a track 13, 15, 17

Cell, **moving** in the direction of the arrow 16

Cell residues in the form of filaments and membrane fragments, left by the cell in (15) which is surface modified 14a,14b.
Dwg.1/7

L95 ANSWER 19 OF 27 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN
ACCESSION NUMBER: 2000-604972 [58] WPIDS
DOC. NO. NON-CPI: N2000-447647
DOC. NO. CPI: C2000-181627
TITLE: Lipoprotein analyzer for analyzing **blood** and cell culture samples, comprises separation column into which sample is introduced through single path and eluted into several paths, attached with a detector.
DERWENT CLASS: B04 D16 **J04 S03**
PATENT ASSIGNEE(S): (TOYJ) TOSOH CORP
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
JP 2000214150 A		20000804	(200058)*		6

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
JP 2000214150 A		JP 1999-16779	19990126

PRIORITY APPLN. INFO: JP 1999-16779 19990126
AB JP2000214150 A UPAB: 20001114
NOVELTY - Lipoprotein analyzer comprises separation column into which biological sample is introduced through single path and eluted into several paths, attached with a detector.
DETAILED DESCRIPTION - Lipoprotein analyzer comprises separation column into which biological sample is introduced through single path and eluted into several paths, attached with a detector. The sample is introduced into a separation column of liquid chromatography apparatus, through a single flow path and the elution component from the separation column is divided and passed through several flow paths, each comprising a detector. Lipoprotein in each component is detected by the detector using reagents capable of reacting with cholesterol, triglycerides and phospholipids.
USE - For analyzing lipoprotein contained in blood, serum, plasma and cell culture supernatant liquids.
ADVANTAGE - Interchanging of reaction reagents is not needed. Similar analysis results are obtained and results can be obtained within short time period using a very small amount of the sample.
DESCRIPTION OF DRAWING(S) - The figure shows the outline of the lipoprotein analyzer of the liquid chromatography apparatus.
Dwg.1/3

L95 ANSWER 20 OF 27 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN
ACCESSION NUMBER: 2000-096776 [08] WPIDS
DOC. NO. CPI: C2000-028033
TITLE: Cell **selection** method, useful for enriching and isolating cells from suspensions .
DERWENT CLASS: B04 D16 K02 K07 K08
INVENTOR(S): EPPICH, H M; REILLY, D A
PATENT ASSIGNEE(S): (SCRE-N) SCI RES LAB INC
COUNTRY COUNT: 86
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG

WO 9954439 A1 19991028 (200008)* EN 61
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZA ZW
AU 9935688 A 19991108 (200014)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9954439	A1	WO 1999-US8512	19990416
AU 9935688	A	AU 1999-35688	19990416

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9935688	A	Based on	WO 9954439

PRIORITY APPLN. INFO: US 1998-103944P 19981013; US 1998-82195P 19980417

AB WO 9954439 A UPAB: 20000215

NOVELTY - A method (I) for selecting cells is new and comprises:

(a) providing a mixture of cell types in a suspension, each cell type including viable cells and having different nuclear volume to total cell volume ratios (R); and

(b) enriching at least a first cell type relative to a second cell type on the basis of the difference of the (R), by changing an osmolarity of the suspension.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a suspension of cells (II) obtained by (I);

(2) a system (III) comprising:

(a) a cell suspension containment element (1) adapted to contain a mixture of at least two cell types in a suspension;

(b) a dispenser (2) adapted to dispense a predetermined quantity of a lysing solution with a predetermined osmolarity into the suspension; and

(c) a controller (3) which determines the predetermined quantity of the lysing solution required to change an osmolarity of the suspension, so that a fraction of a population of viable cells of the first cell type is reduced to a greater extent than a fraction of a population of viable cells of the second cell type. It also actuates the dispenser to deliver the lysing solution to the suspension;

(3) a cell suspension (IV), comprising:

(i) a first population of viable cells with a maximum (R) of at least a predetermined value; and

(ii) a second population of non-viable and/or lysed cells with a maximum (R) no more than the predetermined value. (IV) is obtained by subjecting a precursor viable cell suspension to an osmolarity which renders cells with a (R) below the predetermined value non-viable.

USE - (I) is used for selecting and enriching cells from a suspension containing a mixture of cells (claimed). The method can be used to isolate **trace** numbers of stem **cells** found in bone marrow aspirate, **mobilized** peripheral blood, umbilical cord blood or fetal liver for use in gene therapy for the treatment of blood diseases. (I) is also useful for purging tumor cells from progenitor cell preparations that are required to rescue patients, via autologous stem cell transplants, after chemotherapy or radiation.

ADVANTAGE - The method allows the rapid and cost-effective isolation

of target cells from suspensions containing a diverse mixture of cell types and concentrations. Stem cells can be isolated from any source of animal or human tissue.
Dwg.0/10

L95 ANSWER 21 OF 27 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN
ACCESSION NUMBER: 1997-072249 [07] WPIDS
DOC. NO. NON-CPI: N1997-059937
TITLE: Cell discriminating apparatus - reads image of cell surfaces to **extract** discriminating information.
DERWENT CLASS: P43 X16
PATENT ASSIGNEE(S): (RICO) RICOH KK
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
JP 08318223	A	19961203	(199707)*		9

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
JP 08318223	A	JP 1995-127810	19950526

PRIORITY APPLN. INFO: JP 1995-127810 19950526
AB JP 08318223 A UPAB: 19970212
The apparatus includes a device to move cells, a device to read images of surfaces of cells under movement, a means to extract discriminating information from read images. It discriminates whether cells contain material to be recycled, require attention due to high energy.
A separating means switches transfer **paths** for **cells** under **movement** based on the discrimination results. A device receives the first kind of cells as they are. A device encloses the second kind of cells with an insulating sheet. A second device receives the insulated enclosed cells. A device crushes cells of a third kind and a third device receives the crushed cells.
ADVANTAGE - Post treatment can be made safety and efficient.
Dwg.1/12

L95 ANSWER 22 OF 27 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN
ACCESSION NUMBER: 1987-061701 [09] WPIDS
DOC. NO. NON-CPI: N1987-046575
DOC. NO. CPI: C1987-025997
TITLE: Device for testing **corneal** cells - has means for tracing borders of cells on photographic image and means for calculating area of obtd. polygon and outputting statistical data.
DERWENT CLASS: B04 **J04** P31 **S03 S05**
PATENT ASSIGNEE(S): (KONA-N) KONAN CAMERA KENKYUSHO
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
JP 62017659	A	19870126	(198709)*		12

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
JP 62017659	A	JP 1985-157858	19850716

PRIORITY APPLN. INFO: JP 1985-157858 19850716
AB JP 62017659 A UPAB: 19930922
Device comprises: a **moving** member (e.g., cursor or pen of digitiser) to trace the output line of a photographed cell image; a coordinate output means to output the present coordinates of the **moving** member; a coordinate sampling means to extract a number of coordinates representing the border line by sampling the output data of the output means; a polygonal area calculating means to calculate the area of a polygon obtd. by connecting the coordinates outputted from the sampling means; and a statistic data output means to calculate statistic data from the output data of the polygonal area calculating means and to output it.
ADVANTAGE - Statistic data necessary for diagnosis can be obtd. solely by tracting roughly the border line of each cell.
0/0

L95 ANSWER 23 OF 27 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN
ACCESSION NUMBER: 1986-031805 [05] WPIDS
DOC. NO. NON-CPI: N1986-022989
DOC. NO. CPI: C1986-013284
TITLE: Cell and blood **corpuscle** handling device - includes FETs arranged along charged **cell movement path** to measure **cell** speed.
DERWENT CLASS: B04 D16 **J04 S03 S05**
PATENT ASSIGNEE(S): (HITA) HITACHI LTD
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
JP 60251873	A	19851212	(198605)*		3

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
JP 60251873	A	JP 1984-108356	19840530

PRIORITY APPLN. INFO: JP 1984-108356 19840530
AB JP 60251873 A UPAB: 19930922
In the device to **move** the position of the charged cells etc. or stop their **movement** by controlling the potential or electrode- area of a number of electrodes arranged on a plane; at least two field effect transistors are arranged along the **moving** pass of the charged cells. By measuring the time required for the cells to pass between the two field effect transistors, the **moving** speed of the cells is obtd., and, from the magnitude of the speed, changes in the electric properties of the cells and the buffer soln. can be found. The voltage for electrophoresis of cells is adjusted depending upon their speed.
A cell, etc. that is charge negatively can be **moved** a small distance through electrophoresis by providing a higher potential on one electrode than on the another. It is assumed that cells **move** in a buffer soln. from an electrode to another electrode. Two field effect transistors of PNP type are arranged apart a given distance. When the grain **moves** between the two transistors, the time required to pass between them can be obtd. from the changes in current, and the speed of **movement** is calculated.
USE/ADVANTAGE - For automatic positioning and control of stopping of

movement of cells, blood corpuscles, etc.. Even when the properties of the buffer soln. or characteristics of grains change, the process can be carried out with high accuracy.
0/0B

L95 ANSWER 24 OF 27 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN
ACCESSION NUMBER: 1986-071506 [11] WPIDS
DOC. NO. CPI: C1986-030431
TITLE: Appts. for handling fine particles e.g. cells or blood **corpuscles** - has conductive path moving or stopping charged fine particles.
DERWENT CLASS: D16 J04
PATENT ASSIGNEE(S): (HITA) HITACHI LTD
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
JP 60248163	A	19851207	(198611)*		6

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
JP 60248163	A	JP 1984-104554	19840525

PRIORITY APPLN. INFO: JP 1984-104554 19840525
AB JP 60248163 A UPAB: 19930922
Fine particle handling, equipment has conductive path moving or stopping charged fine particles. Electrodes are alternately provided on facing walls of conductive path. Moving or stopping fine particles is controlled by applying voltage to electrodes.
USE/ADVANTAGE - Method automatically moves or stops fine particles.
0/0

L95 ANSWER 25 OF 27 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN
ACCESSION NUMBER: 1983-801612 [44] WPIDS
DOC. NO. NON-CPI: N1983-192542
DOC. NO. CPI: C1983-104876
TITLE: **Laboratory** ware for searching and counting - has interconnected broken ring markings forming single spiral scan path.
DERWENT CLASS: **J04 S03**
INVENTOR(S): BERJAULT, R
PATENT ASSIGNEE(S): (BERI-I) BERIAULT R
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
CA 1154614	A	19831004	(198344)*		8

PRIORITY APPLN. INFO: CA 1981-381093 19810703
AB CA 1154614 A UPAB: 19930925
Vessel, slide, plate or filter has markings forming a spiral path (8) originating at or near the centre (9) and formed by a series of concentric rings (6), broken and linked together to form an outwardly extending continuous **path**. The **path** pref. has dividing lines to facilitate searching for minute embryos used for embryo transfer, or for counting other structures, organisms or particles.

The path pref. has symbols, numerals or letters at intervals to act as ref. points. The path may be defined by an upward wall, or by etched, printed or embossed lines, or lines created by incorporating a different material into the basic material. Arrangement eliminates the need to scan back and forth along straight lines by providing a single line and direction of movement to minimise the danger of inaccuracy.
1/8

L95 ANSWER 26 OF 27 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN
ACCESSION NUMBER: 1982-17859E [09] WPIDS
TITLE: **Harvesting** microorganisms, esp. free swimming unicellular **algae** - by providing **path** for **cell migration** from cell reservoir to **harvesting** zone using fibrous or inert inorganic material.
DERWENT CLASS: D16 P13
INVENTOR(S): KESSLER, J O
PATENT ASSIGNEE(S): (UYAR-N) UNIV ARIZONA FOUNDATION
COUNTRY COUNT: 3
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
ZA 8007239	A	19811002	(198209)*		33
US 4324067	A	19820413	(198217)		
IL 61505	A	19831130	(198404)		

PRIORITY APPLN. INFO: US 1980-118585 19800204; US 1982-347550 19820210
AB ZA 8007239 A UPAB: 19930915
The product yield of algal cells, pref. of the Dunaliella genus, is increased. The cells are disposed in a saline liquid-contg. reservoir for multiplication by cell division. A portion of the cells are induced to move by capillary motion from the reservoir to a concn. harvest zone. Liquid is evaporated from the harvest zone to increase the salinity of the remaining liquid in the harvest zone and thus increase the product content of the cells.
The system is used for harvesting mobile swimming microorganisms, particularly of the Dunaliella genus for the prodn. of glycerol.

L95 ANSWER 27 OF 27 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN
ACCESSION NUMBER: 1973-27898U [20] WPIDS
TITLE: Formatn rates and cell migration determinatn - by using hydrogen and carbon **isotopes**.
DERWENT CLASS: B04 K08 S03
PATENT ASSIGNEE(S): (GRA-I) GRACHEVA ND
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
SU 352179	A		(197320)*		

PRIORITY APPLN. INFO: SU 1970-1447205 19700608
AB SU 352179 A UPAB: 19930831
Formation rates and **migration paths** of **cells** are determined by introducing H3-thymidine into a pregnant animal and additionally introducing C14-thymidine into the pregnant animal at a time interval between the administrations of the H3-thymidine and equal to the

generative cycle, followed by a study of the 'autographs' of histological sections obtd. from the descendants at a certain age from the time of birth.

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INTERNATIONALE ANMELDUNG VERÖFFENTLICHT NACH DEM VERTRAG ÜBER DIE INTERNATIONALE ZUSAMMENARBEIT AUF DEM GEBIET DES PATENTWESENS (PCT)

(51) Internationale Patentklassifikation 7 : G01N 33/483, C12N 5/00	A1	(11) Internationale Veröffentlichungsnummer: **WO 00/36415** (43) Internationales Veröffentlichungsdatum: 22. Juni 2000 (22.06.00)

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(22) Internationales Anmeldedatum: 10. Dezember 1999 (10.12.99)

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198 57 692.7 14. Dezember 1998 (14.12.98) DE

(71) Anmelder *(für alle Bestimmungsstaaten ausser US)*: EVOTEC BIOSYSTEMS AG [DE/DE]; Schnackenburgallee 114, D–22525 Hamburg (DE).

(72) Erfinder; und
(75) Erfinder/Anmelder *(nur für US)*: FUHR, Günter [DE/DE]; Kavalierstrasse 15, D–13187 Berlin (DE). HAGEDORN, Rolf [DE/DE]; Wartiner Strasse 16, D–13057 Berlin (DE). SHIRLEY, Stephen, Graham [GB/GB]; Victoria Main Street, Brandon, Warwickshire CV8 3NW (GB). RICHTER, Ekkehard [DE/DE]; Walkürenstrasse 3A, D–10318 Berlin (DE).

(74) Anwalt: HERTZ, Oliver; v. Bezold & Sozien, Akademiestrasse 7, D–80799 München (DE).

(81) Bestimmungsstaaten: JP, US, europäisches Patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

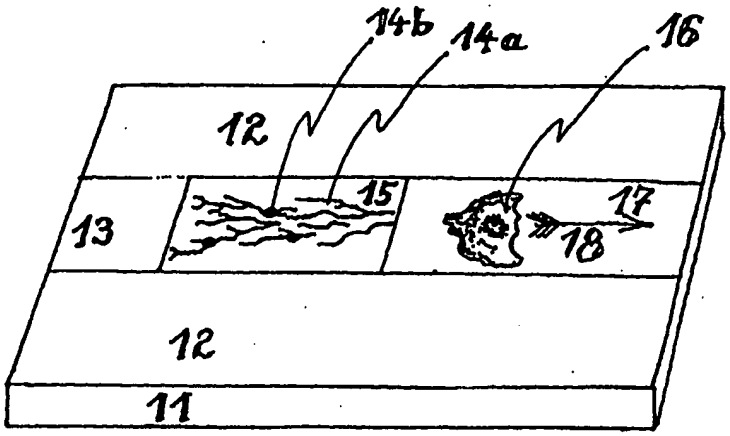
Veröffentlicht
Mit internationalem Recherchenbericht.

(54) Title: METHOD AND DEVICE FOR THE CELL–TRACK–BASED EXAMINATION AND CULTIVATION OF CELLS

(54) Bezeichnung: VERFAHREN UND VORRICHTUNG ZUR ZELLSPURBASIERTEN ZELLUNTERSUCHUNG UND ZELLKULTIVIERUNG

(57) Abstract

The invention relates to a method for the cell–track–based examination of biological cells, according to which the cells (16) are applied to an at least partly structured and/or surface–modified substrate (11) and move across track areas (13, 15) of the surface of the substrate in an adhesive manner such that they generate cell tracks (14a, 14b) which consist of material residues separated by the cells. The cells are then studied on the basis of these tracks. The invention further relates to a method for cultivating cells on substrates modified in a biocompatible manner whose surfaces are covered in cell tracks.



(57) Zusammenfassung

Verfahren zur zellspurbasierten Untersuchung biologischer Zellen, bei dem die Zellen (16) auf ein zumindest teilweise strukturiertes und/oder oberflächenmodifiziertes Substrat (11) aufgebracht werden und sich adhärent über Bahn–Oberflächenbereiche (13, 15) des Substrats unter Erzeugung von Zellspuren (14a, 14b) bewegen, die aus von den Zellen abgetrennten Materialrückständen bestehen, werden Zelluntersuchungen an den Zellspuren durchgeführt. Es wird auch ein Verfahren zur Zellkultivierung auf biokompatibel modifizierten Substraten beschrieben, deren Oberflächen von Zellspuren bedeckt sind.

LEDIGLICH ZUR INFORMATION

Codes zur Identifizierung von PCT-Vertragsstaaten auf den Kopfbögen der Schriften, die internationale Anmeldungen gemäss dem PCT veröffentlichen.

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Verfahren und Vorrichtung zur zellspurbasierten Zelluntersuchung und Zellkultivierung

Die Erfindung betrifft Verfahren und Vorrichtungen zur Zelluntersuchung, insbesondere Zellassays oder Zell-Testanordnungen, deren Herstellung und Verfahren zu deren Verwendung. Die Erfindung betrifft auch Verwendungen von Zellspuren auf Substratoberflächen.

In der Pharmakologie, Toxikologie und medizinischen Diagnostik nehmen zellbasierte Assays (Reaktionsansätze, Prüfansätze o. dgl.) eine Schlüsselstellung ein. Gesucht werden rasch handhabbare und hochspezifische Untersuchungs- und Nachweisverfahren für biologische Zellen an sich oder für deren Wechselwirkungen mit anderen Zellen oder mit natürlichen oder synthetischen Fremdsubstanzen. Darüber hinaus wäre es wünschenswert, wenn die zum Nachweis einer Substanz, eines Zelltyps, einer Medikamentwirkung etc. benutzten Zellen wiederverwendbar (d.h. weiter kultivierbar) wären. Im medizinischen Bereich bedeutet das z.B. eine Rückführung in den Organismus des Spenders (z. B. eines Patienten) oder eines anderen menschlichen Empfängers. Für derartige Prozeduren werden jedoch neben der Sterilität sehr hohe Anforderungen daran gestellt, daß sich die Zelleigenschaften durch die Untersuchungen oder Analyse nicht verändern. So ist eine Markierung (z.B. für die Fluoreszenzerzeugung), wie sie bei der Immunofluorochromierung verwendet wird, ausgeschlossen, da die Folgereaktionen derart kontaminierter Zellen bei einer nachfolgenden Kultivierung oder im Empfängerorganismus nur schwer abschätzbar sind.

Es besteht ferner ein Interesse daran, Zelluntersuchungen spezifisch an Einzelzellen durchzuführen. Für ein statistisch ab-

gesichertes Untersuchungsergebnis muß eine genügend hohe Zahl von Einzelzellen untersucht werden. Daraus ergibt sich ein Bedarf an bisher nicht verfügbaren Paralleluntersuchungen an einer Vielzahl von Einzelzellen unter möglichst gleichartigen Bedingungen.

Die Aufgabe der Erfindung besteht darin, extrem belastungsarme, auch für den medizinischen Bereich einsetzbare Verfahren und Vorrichtungen zur Zelluntersuchung zu entwickeln, die mit möglichst vielen bereits erprobten hochspezifischen Nachweistechniken, wie z.B. Immunofluorochromierung von Proteinen, Nukleotiden und Lipiden, als auch zerstörenden Verfahren wie z.B. Röntgen- oder Elektronenstrahlmikroanalyse kombiniert werden können und die parallele Untersuchung einer Vielzahl von Einzelzellen erlauben. Die Aufgabe der Erfindung ist es auch, neue Anwendungen von Zellspuren anzugeben.

Diese Aufgabe wird durch Verfahren und Vorrichtungen mit den Merkmalen gemäß den Patentansprüchen 1 bzw. 17 gelöst. Vorteilhafte Ausführungsformen und Verwendungen der Erfindung ergeben sich aus den abhängigen Ansprüchen.

Die Grundidee der Erfindung besteht insbesondere darin, zur Untersuchung oder -kultivierung von biologischen Zellen oder deren Wechselwirkungen mit anderen Zellen oder Substanzen zunächst auf einer Substratoberfläche Zellspuren der zu untersuchenden Zellen zu erzeugen und dann diese Zellspuren der gewünschten Analyse oder Untersuchung zu unterziehen. Die Erzeugung von Zellspuren durch adhärent auf Oberflächen wachsende oder sich bewegende Zellen ist an sich bekannt und wird beispielsweise von E. D. Hay et al. in "Exp. Biol. Med.", Bd. 10, 1985, S. 174 ff., beschrieben. Die Strukturen und Eigenschaften von Zellspuren werden unten unter Bezug auf die Figuren 2 und 3 erläutert. Die Erzeugung der Zellspuren erfolgt vorzugsweise unter Verwendung von Substratoberflächen, die zu-

mindest teilweise in geeigneter Weise mikrostrukturiert und/oder modifiziert sind. Die Mikrostrukturierung der Substratoberfläche ist insbesondere dazu vorgesehen, die Zellspurerzeugung in bestimmten Substratbereichen, z.B. entlang bestimmter Pfade, zu fördern und in anderen Substratbereichen zu behindern oder auszuschließen. Die Oberflächenmodifizierung führt ferner dazu, daß nicht nur die auf natürliche Weise auf dem Substrat zurückbleibenden Materialspuren untersucht werden können, sondern auch künstlich abgetrennte Materialrückstände (Erzielung größerer Spurmengen). Hierzu umfaßt die Substratmodifizierung insbesondere die Aufbringung von Molekülen, die Bindungsstellen anbieten, an denen spezifisch ein vorbestimmtes, gesuchtes Zelloberflächenmolekül ankoppeln kann.

Die erfindungsgemäß eingesetzten Untersuchungsmethoden umfassen alle an sich zur Zelluntersuchung und Zellbehandlung bekannten Techniken, wobei sowohl zerstörungsfreie als auch zerstörende Techniken oder gegebenenfalls biochemische Verstärkungstechniken (z.B. PCR-Prozeß) eingesetzt werden können.

Gegenstand der Erfindung ist auch die Verwendung von Zellspuren zur Manipulierung der Wechselwirkung biologischer Zellen mit festen Substraten. Durch gezieltes Aufbringen von Zellspuren auf synthetischen oder biologischen Substraten entsprechend den in der vorliegenden Beschreibung erläuterten Prinzipien werden biokompatible Träger für die zu manipulierenden biologischen Zellen bereitgestellt. Die Manipulation besteht insbesondere in der gezielten Zellkultivierung (Gewebeaufbau) auf den mit Zellspuren belegten Substratbereichen.

Die erfindungsgemäß verwendeten Substratoberflächen können aus synthetischem, anorganischem oder organischem Material bestehen oder auch durch biologisches Material (z.B. Knochenmaterial) gebildet werden.

Die Erfindung besitzt die folgenden Vorteile. Es wird erstmalig ein einzelzellspezifisches Verfahren zur Zelluntersuchung angegeben, bei dem die untersuchte Zelle durch den Untersuchungsvorgang unbeeinflußt und unverändert bleibt. Dies erlaubt eine erhebliche Erweiterung der Anwendung von Einzelzelluntersuchungen in der Pharmakologie, Toxikologie, medizinischen Diagnostik und Biochemie. Die Zelluntersuchung kann hochgradig parallel an einer Vielzahl von Zellen durch gleichzeitige Erzeugung vieler Spuren auf einem Substrat durchgeführt werden. Da durch die Mikrostrukturierung der Oberflächen eine Zuordnung einer Zellspur zu einer untersuchten Zelle (Spenderzelle) möglich ist, bleibt die hochparallele Einzelzelluntersuchung ebenfalls zellspezifisch. Die Mikrostrukturierung der Substratoberfläche kann vorzugsweise nach Techniken erfolgen, wie sie an sich aus der Halbleiterprozessierung bekannt sind.

Erfindungsgemäß werden die Zellen außer durch die Spurenerzeugung durch keinerlei Färbungs- oder Markierungstechniken belastet. Sie sind damit nicht kontaminiert oder verändert und können einer medizinischen Verwendung bzw. einer Kryokonservierung oder einer weiteren Kultivierung unterworfen werden. Statt wie bisher in Zellen werden die Zellrückstände einer spezifischen Markierung oder Bewertung unterworfen. Diese kann durchaus auch zerstörend sein (z.B. schrittweiser enzymatischer Abbau), oder über Immunofluorochromierung in toxischen Konzentrationsbereichen erfolgen.

Die erfindungsgemäße Zellkultivierung besitzt den Vorteil, daß mit Zellspuren beliebige Substratmaterialien, wie sie beispielsweise für die Implantation von Knochenmaterialien von Interesse sind, biokompatibel gemacht werden können. Es werden neuartige Substrate zum in-vitro-Gewebeaufbau geschaffen, die einen erheblich erweiterten Anwendungsbereich besitzen. Durch die zellspurbasierte Modifikation von Substratoberflächen können anwendungsabhängig optimierte Zellkulturen auf optimierte

Substratmaterialien aufgebracht werden, die ohne die Zellspuren gegebenenfalls nicht miteinander kompatibel wären.

Weitere Ausführungsbeispiele und Vorteile der Erfindung werden im folgenden unter Bezug auf die beigefügten Zeichnungen beschrieben. Es zeigen

Fig. 1 den prinzipiellen Aufbau eines erfindungsgemäßen zellspurbasierten Systems (Ausschnitt),

Fig. 2 eine schematische Illustration der Grundstrukturen von Zellspuren in Form von Filamenten (A) und Membranflecken (B),

Fig. 3 eine Illustration der Wirkungsweise eines modifizierten Substrats,

Fig. 4 eine Illustration zur erfindungsgemäßen Fluoreszenzuntersuchung von Zellspuren,

Fig. 5 ein Ausführungsbeispiel der Erfindung, bei dem das in Fig. 1 illustrierte Grundprinzip mit einer Vielzahl paralleler Bahnen realisiert ist,

Fig. 6 ein weiteres Ausführungsbeispiel der Erfindung mit einer Vielzahl paralleler Zellbahnen, und

Fig. 7 ein weiteres Ausführungsbeispiel der Erfindung mit sich kreuzenden Zellbahnen.

In Fig. 1 ist der prinzipielle Aufbau eines erfindungsgemäßen zellspurenbasierten Systems dargestellt. Ein Substrat 11 ist in seiner Oberfläche im µm- und mm-Bereich wie folgt strukturiert bzw. in seinen Oberflächeneigenschaften verändert.

Durch Bereiche 12 der Oberfläche, an denen Zellen nur schlecht adhärieren können, und Bereiche 13, 15, 17 (Bahn-Oberflächenbereiche), wo Zellen gut anhaften können, wird eine Vorzugsbahn gebildet, auf der sich eine Zelle 16 aktiv bewegen kann. Das Feld 15 im Bahn-Oberflächenbereich ist so modifiziert worden (chemisch, mechanisch etc.), daß hier die Zellen Teile ihrer Membran und inneren Bestandteile 14a, 14b verlieren, die am Substrat anhaften. Im gezeigten Beispiel sind es Filamente 14a und Membranflecken (oder Membranpatches) 14b, die unten unter Bezug auf die Fig. 2A und 2B im einzelnen erläutert werden. Die Zelle bewegt sich weiter in Richtung des Pfeiles 18. Die Zellspur kann nunmehr zerstörungsfrei oder zerstörend analysiert werden. Das zurückgelassene Material charakterisiert die Spenderzelle hinsichtlich der Membranzusammensetzung (Rezeptoren, Carrier, Lipide usw.), aber auch hinsichtlich innerer Bestandteile des Zytoplasmas, woraus sich medizinische, toxikologische, pharmakologische und andere Anwendungen ableiten lassen. Auf einer Bahn kann sich entweder eine oder mehrere Zellen bewegen und Spuren erzeugen.

Das Substrat 11 besteht beispielsweise aus Glas, Glimmer, anorganischem Kristallmaterial oder Halbleitermaterial. Die Substratoberfläche ist einerseits zur Ausbildung der Vorzugsbahn strukturiert bzw. modifiziert, auf der sich die Zelle bevorzugt bewegt und Zellspuren hinterläßt. Die Oberflächenbereiche 12, an denen Zellen nur schlecht adhärieren können, tragen beispielsweise eine Beschichtung mit negativ geladenen Molekülen, vorzugsweise aus Polymeren mit möglichst vielen OH^--Gruppen, wie z. B. Poly-HEMA. Beispiele für die Beeinflussung der Bereiche 13, 15, 17, in denen die Zellen gut anhaften können, werden unten gegeben. Andererseits umfaßt die Mikrostrukturierung und/oder Modifizierung der Substratoberfläche eine örtlich selektive Beeinflussung der Vorzugsbahn zwischen den Bereichen 12 der Substratoberfläche. Die Segmentierung der Vorzugsbahn z.B. in die Bereiche 13, 15 und 17 ist dazu vorgesehen, daß je nach

der Gestaltung des jeweiligen Bereiches die Zellspuren besonders zahlreich oder besonders gering oder in Bezug auf eine bestimmte Zusammensetzung zurückgelassen werden. Dies wird auch aus den unten erläuterten Beispielen ersichtlich.

Die Mikrostrukturierung bzw. Modifizierung der Vorzugsbahn umfaßt beispielsweise:

1. Aufbringen von den molekularen Zellkontakt erhöhenden Schichten (z.B. Fibronektin, Polylysin, Alginate etc.). Die Schichtdicke kann anwendungsabhängig von der Dike einer Moleküllage bis hin in den µm-Bereich gewählt werden. Die Molekülmonolagen werden vorzugsweise mit der Langmuir-Blodget-Technik aufgebracht. Generell sind zur Schichtaufbringung auch Dickschichttechniken und/oder Plasmabehandlungen einsetzbar.

2. Nano- bzw. Mikrostrukturisierung von Oberflächen, d.h. Aufbringen von Mustern in nm- bzw. µm-Dimensionen, an denen Membranteile, insbesondere aber natürliche Kontaktmoleküle der Zelle, wie die der Integrin- und Catherinfamilie anhaften können (z.B. Strukturierung über die Photo- oder Elektronenstrahl-Lithographie.

3. Submikrometer und atomare Aufrauhung oder Reliefbildung auf Oberflächen (kleinste Widerhaken etc.).

Die Substratbeschickung (Aufbringung der Zellen) erfolgt beipielsweise durch Aufspülen aus einer Suspension, beispielsweise durch einen Kanal des Mikrosystems, mit einem Manipulator (Kapillare, separates Mikrosystem oder optische Pinzette) oder auch durch aktives Aufwachsen.

Bei der Wanderung der Zellen über Substratoberflächen (z.B. über eine saubere Glasoberfläche) hinterlassen die Zellen unter

physiologischen Bedingungen filamentöse oder fleckenartige Spuren, die im folgenden als Filament bzw. Membranfleck bezeichnet und unter Bezug auf die Fig. 2A bzw. 2B erläutert werden. Die Spuren sind in der Regel Strukturen, die membranumhüllt und mit Zellinhalten gefüllt sind. Typische Größen dieser Strukturen liegen in Bezug auf die Breite und Höhe im µm- und Sub-µm-Bereich. Während die Länge eines Membranflecks in der Regel im wesentlichen seiner Breite entspricht, ist die Länge eines Filaments variabel. Die Filamentlänge kann bis zu einige Millimeter betragen. Die interessierenden Bestandteile der Zellen, die auch in den Zellspuren auffindbar sind, sind Membranproteine 210, Oberflächenproteine und -rezeptoren 211, 212, Zytoplasmabestandteile 213, 214 und die Lipide 215 in der Membran (s. Fig. 2A). Bei den Membranflecken treten neben diesen Bestandteilen, die in Fig. 2B z.B. die Membranproteine 220 und die Lipidzusammensetzung 225 umfassen, ferner Vesikeln 221, Organellen 222 und genetisches Material 223 auf. Außerdem ist auch Zytoplasma 224 vorhanden. Im Rahmen der vorliegenden Erfindung wurde erstmalig festgestellt, daß die Zellspuren analysefähiges Material, das u.a. die genannten Bestandteile umfaßt, in ausreichender Menge enthalten. Dies bedeutet, daß die an sich bekannten Analyse- oder Untersuchungsverfahren vorteilhafterweise ohne gesonderte Anreicherungsschritte implementiert werden können.

Die Oberflächenproteine und -rezeptoren 211, 212 umfassen beispielsweise ein Spurenprotein 211 in der Membran und einen angekoppelten Rezeptor 212 mit einer chromophoren Gruppe. Vom Rezeptor 212 wird bei geeigneter Lichtanregung Fluoreszenzlicht ausgestrahlt, das das Vorhandensein des Spurenproteins 211 anzeigt. Da die Rezeptorankopplung proteinspezifisch erfolgt, kann mit dem Fluoreszenzlicht der in der Spur vorhandene Proteinkomplex nachgewiesen werden.

In analoger Weise lassen sich auch andere Nachweistechniken implementieren, wie sie beispielsweise bei den ELISA- und RIAS-Assays vorgesehen sind.

Erfindungsgemäß erfolgt somit an den Zellspuren der spezifische Nachweis bestimmter Bestandteile der Spenderzelle. Die Bestandteile können auf der Oberfläche oder im Inneren der Zellspuren angeordnet sein. Im letzteren Fall ist vorgesehen, die Membran der Zellspur mit geeigneten Lösungsmitteln aufzulösen oder mechanisch oder elektrisch zu permeieren. Die zerstörende Messung an den Zellspuren ohne Veränderung der Spenderzelle zur Erfassung von molekularen oder mikroskopischen Bestandteilen in den Zellspuren stellt einen besonderen Vorteile der Erfindung dar.

Die zellspurbasierten Analysen sind in besonderer Weise zur Kombination mit hochempfindlichen Meßtechniken geeignet. Diese umfassen beispielsweise die Fluoreszenz-Korrelationsanalyse zum Einzelmolekülnachweis und zur Bestimmung von Bindungskonstanten, die Massenspektrometrie zur Elementaranalyse und die konfokale Laserscanningmikroskopie. Genetisches Material in den Spuren kann z.B. über einen PCR-Prozeß amplifiziert werden, wodurch eine neuartige, die Spenderzelle in ihren physiologischen Zellen nicht beeinflussende Technik einer genetischen Analyse gegeben ist.

Für einzelzellbasierte Assays und Nachweisverfahren können auch die folgenden Prozeduren angewendet werden.

1. Die Menge der Zellrückstände wird als quantitatives Maß für die Stärke des Anhaftens der Spenderzelle an der Substratoberfläche und somit für die Menge bestimmter Bindungskomplexe in deren Membran erfaßt.

2. Die Spurenstruktur wird als Maß erfaßt, z.B. die Verhält-

nisse des Anteils an Filamenten zum Anteil an Verzweigungen, zum Anteil an Patches usw. (Vergleich von Zellspurgrundelementen in ihrer Quantität).

3. Die Materialzusammensetzung der Spuren wird als Maß erfaßt, z.B. Lipid/Proteinanteil, spezifisches Auftreten bestimmter Rezeptoren (u.a. der Immunglobulinfamilien), spezifisches Auftreten von Lipiden, Nukleotiden etc.

4. Charakterisierung zytoplasmatischer Rückstände, insbesondere genetischen Materials in den Rückständen der Zellen.

5. Vergleich von Änderungen in einem der Punkte 1 bis 4 nach Behandlung der spurerzeugenden Zelle (z.B. mit Pharmaka, toxischen Substanzen etc.).

6. Die Stabilität der Spur gegen mechanische, elektrische, akustische, optische oder chemische Behandlungen wird als Maß erfaßt.

7. Die Elementzusammensetzung der Spuren oder Teile derselben (z.B. Na, K, P...) werden als Maß erfaßt.

8. Passive elektrische Parameter der Zellrückstände, wie Impedanz, Durchschlagsfestigkeit, nichtlineares Verhalten oder Erwärmung werden als Maß erfaßt.

9. Optische Parameter der Spuren werden als Maß erfaßt, wie Absorption, Transmission, nichtlineare Eigenschaften etc.

10. Mechanische Eigenschaften der Spuren, wie Elastizität, Plastizität usw. werden als Maß erfaßt.

11. Die Veränderung einer Zellspur durch eine nachfolgende Zelle der gleichen oder einer anderen Art wird als Maß

erfaßt.

12. Die Spurcharakterisierung erfolgt nach einer Fixierung bzw. Kontrastierung, z.B. mittels hochauflösender Mikroskopverfahren (Rasterelektronenmikroskopie, AFM, SNOM etc.).

13. Ein Negativ oder anderes Duplikat oder eine Vervielfachung von Spurenteilen wie bei der PCR-Technik wird als Maß für den Vergleich benutzt.

14. Die Adhäsion anderer Materialien, wie hochspezifisch bindender Beads oder nm-Teilchen werden als Maß für den Vergleich erfaßt.

Die hier genannten Maße werden als für die Spenderzellen spezifischer Größen erfaßt, die bei gegebenen Vergleichswerten eine Charakterisierung der Spenderzelle oder bei Vergleich der Maße mit den entsprechenden Ergebnissen bei anderen Zellen zur Charakterisierung des unterschiedlichen Verhaltens der Zellen verwendet werden.

Im folgenden werden unter Bezug auf die Fig. 3 bis 7 Ausführungsformen erfindungsgemäßer Vorrichtungen erläutert. Dabei wird auf Einzelheiten der erfindungsgemäß eingesetzten Substratoberflächen eingegangen. Nicht dargestellt sind Einzelheiten einer Gesamtapparatur zur Zellspuruntersuchung, da diese in Bezug auf die Handhabung von Assays bzw. von mit Proben beschickten Substraten und die Anpassung an die jeweils gewünschten Untersuchungsmethoden an sich bekannt sind.

Die Fign. 3 und 4 sind schematische Schnittansichten von Substraten 31, 41, die jeweils mit einer Modifizierungsschicht 32 bzw. 42 im Bahn-Oberflächenbereich 35 bzw. 45 zur bevorzugten Anhaftung von Zellen und Hinterlassung von Zellspuren 34 bzw. 44 ausgestattet sind. Die Modifizierungsschicht 32 (bzw. 42)

bietet Bindungsstellen an, an denen spezifisch ein vorbestimmtes, gesuchtes Zelloberflächenmolekül 33 (bzw. 43) ankoppeln kann. Eine Zelle, die derartige Moleküle in ausreichender Zahl besitzt, wird dementsprechend fester gebunden sein und mehr Spurenmaterial 34 (bzw. 44) bei ihrer Wanderung über das Substrat zurücklassen als andere Zelltypen. Die Erfassung der Menge des zurückgelassenen Materials (z.B. mit optischen Mitteln) liefert eine Aussage über den Gehalt des vorbestimmten Zelloberflächenmoleküls an der untersuchten Zelle. Die Zelle selbst wird durch die Messung nicht belastet.

Falls die Menge des Zellspurmaterials zu gering für eine sichere direkte Auswertung ist, so kann die Zellspurvermessung gemäß Fig. 4 modifiziert sein. Nach der Erzeugung der Zellspuren 44 werden diese mit der Lösung eines Fluoreszenzmarkers behandelt, der als Markermolekül 46 z.B. in die Lipidteile der Zellspur 44 unspezifisch eingebaut wird. Bei geeigneter Lichtanregung und Fluoreszenzmessung kann aus der Intensität des Fluoreszenzlichts auf die Zahl der Markermoleküle 46 und damit auf die quantitative Menge des Zellspurmaterials 44 rückgeschlossen werden.

Die Vorzugsbahn gemäß Fig. 1 bzw. der modifizierte Bahn-Oberflächenbereich 35 (oder 45) gemäß den Fig. 3 (oder 4) können einfach oder mehrfach mit den verschiedensten Geometrien auf dem Substrat ausgebildet sein. Im folgenden werden gerade Vorzugsbahnen beschrieben. Es sind jedoch bei geeigneter Substratstrukturierung auch gekrümmte (z.B. kreisförmige) Vorzugsbahnen möglich.

Fig. 5 zeigt ein Beispiel für eine parallele Ausführung des in Fig. 1 erläuterten Grundprinzips mit einer Vielzahl parallel verlaufender, gerader Vorzugsbahnen.

Auf einem Substrat 51, das z.B. aus Glas, Silizium oder Kunststoff bestehen kann, sind die Zelladhäsion unterbindende Materialien 52 aufgebracht.

Dadurch entstehen eine Vielzahl von Bahnen 57 (Bahn-Oberflächenbereiche), auf denen sich Zellen adhärent bewegen können. Die Bahnen 57 reichen von Eingangsdepots 53 über Oberflächenfelder 55 bis hin zu Ausgangsdepots 58. Die Oberflächenfelder 55 sind so behandelt, daß bevorzugt Zellspuren erzeugt werden. Erreichen die Zellen die ebenfalls für die Adhäsion präparierten Ausgangsdepots 58, so werden sie dort festgehalten bzw. entnommen, um einer Kultivierung, Kryokonservierung oder einer anderen Prozedur unterzogen zu werden. Die Analyse der Spur kann mit allen gängigen Mikronachweisverfahren erfolgen (Fluoreszenz, Isotopenmarkierung, Elementanalyse etc.). Die Oberflächenfelder 55 mit den Zellspuren (nicht dargestellt) sind als Segmente der Bahnen 57 als Reihe, jeweils mit dem gleichen Abstand von dem jeweiligen Eingangsdepot 53 angeordnet. Dies erleichtert die parallele, simultane Auswertung der Zellspuren.

In Fig. 6 ist eine Substratoberfläche in Form eines Mikrosystems gezeigt, die in ähnlicher Weise in verschiedene Oberflächenbereiche kompartimentiert wurde, wie das in Fig. 5 der Fall ist. Das Substrat besteht hier jedoch entweder aus zwei Teilen 61a, 61b oder einem Teil mit einer Sollbruchstelle 61c. Auf den Feldern 65 hinterlassen die Zellen Spuren. Sind sie auf ihrer Wanderung über das Substrat in den Ausgangsdepots 68 angekommen, so wird das Teil 61b entfernt oder abgebrochen. Wie im linken Teil von Fig. 6 gezeigt ist, befinden sich dann die Spuren und Zellen auf jeweils getrennten Substraten 31 und 32, so daß sie auf verschiedene Weise weiterbehandelt werden können.

In Analogie zu diesen Ausführungen lassen sich Oberflächen mit weitaus mehr Zellwegen erzeugen, in denen parallel die Spuren

vieler Zellen so, daß sie eindeutig zuzuordnen sind, erzeugt und charakterisiert werden können.

Fig. 7 zeigt eine Substratoberfläche, die derart strukturiert ist, daß sich zwei Vorzugsbahnen, die für die Wanderung der Spenderzellen eingerichtet sind, kreuzen. Auf dem Substrat 71 verlaufen die Bahnen 77a und 77b im wesentlichen senkrecht zueinander. Im Kreuzungsbereich 75 ist eine Oberflächenmodifizierung oder -strukturierung zur Förderung des Anhaftens von Zellspuren angebracht, wie sie oben erläutert wurde. Außerhalb der Bahnen 77a bzw. 77b ist das Substrat 71 so strukturiert, daß dort keine Zellwanderung stattfindet und keine Zellspuren anhaften.

Mit der in Fig. 7 gezeigten Anordnung lassen sich vorzugsweise Wechselwirkungen zwischen verschiedenen Zellen untersuchen. Es kann z.B. vorgesehen sein, daß zunächst die Zelle 76a über den Bereich 75 wandert und dort Zellspuren hinterläßt. Anschließend wandert die Zelle 76b über denselben Bereich 75 mit den vorhandenen Zellspuren. Mit einem optisch-mikroskopischen Verfahren oder einem anderen Untersuchungsverfahren wird danach erfaßt, ob die Spuren der ersten Zelle 76a durch die zweite Zelle 76b verändert, überlagert oder entfernt wurden. Es kann ferner erfaßt werden, ob geometrische Korrelationen zwischen den Zellspuren auftreten, d.h. ob die Nachfolgezellen den Spuren der Vorgängerzellen folgen oder diese gerade meiden. Daraus lassen sich wiederum zellbasierte Assays für die Medizin, Biotechnologie und Pharmazie mit hoher Spezifik entwickeln. Ein Substrat mit gekreuzten Bahnen läßt sich wiederum zur Erzielung einer Parallelverarbeitung vielfach auf einem gemeinsamen Träger ausbilden.

Die Herstellung eines erfindungsgemäßen Substrats erfolgt vorzugsweise derart, daß zunächst ein Trägermaterial mit einer Beschichtung versehen wird, die für die Zellwanderung und

-adhäsion ungünstig ist (z.B. stark negativ geladene Moleküle). Anschließend wird diese Beschichtung entsprechend dem gewünschten Verlauf der Vorzugsbahnen durch Abtragen strukturiert, so daß das Trägermaterial entsprechend bestimmter geometrischer Formen frei liegt, die dann die Vorzugsbahnen bilden. Anschließend erfolgt anwendungsabhängig die Segmentierung der Vorzugsbahn, d.h. die Aufbringung einer Strukturierung und/oder Modifizierung des Trägermaterials zur verstärkten Zelladhäsion.

Die Bahnbreiten sind vorzugsweise an die charakteristische Ausdehnung einer adhärierten Zelle angepaßt und betragen rd. 50 µm. Die Bahnlängen können ebenfalls anwendungsabhängig ausgewählt werden. Sie betragen z.B. 3 bis 4 charakteristische Zelldurchmesser (d.h. rd. 150 bis 200 Mikrometer) bis hin zu größeren Längen im Millimeterbereich.

Gemäß einer weiteren Ausführungsform der Erfindung erfolgt eine Aufbringung von Zellspuren auf Substraten zur Zellkultivierung. Als Substrate werden ebene oder anwendungsabhängig gekrümmt geformte Festkörpermaterialien synthetischen oder biologischen Ursprungs verwendet. Es werden beispielsweise Glas-, Keramik- oder Kunststoffmaterialien oder auch polierte Knochenscheiben als Substrat verwendet. Um die jeweiligen Substratoberflächen mit einem biokompatiblen Überzug zu versehen, werden nach den oben erläuterten Prinzipien gewebeerzeugende Zellen, wie z.B. Chondrozyten, Osteoplasten oder Epithelzellen auf die Substratoberflächen aufgesetzt, um auf dieser unter Hinterlassung von Zellspuren zu wandern. Die Substratoberfläche kann zur Aufbringung möglichst umfangreicher Zellspuren strukturiert oder anderwertig modifiziert sein (s. oben). Nach Ausbildung einer geschlossenen Zellspuroberfläche erfolgt auf dem modifizierten Substrat ein Gewebeaufbau. Gewebeerzeugende Zellen, vorzugsweise des Typs, mit dem die Zellspuren erzeugt wurden, werden auf dem modifizierten Substrat kultiviert. Ein

Substrat mit kultivierten Gewebezellen wird dann anwendungsabhängig als Implantat in den menschlichen Körper eingesetzt.

PATENTANSPRÜCHE

1. Verfahren zur zellspurbasierten Untersuchung biologischer Zellen, bei dem die Zellen (16, 76a, 76b) auf ein zumindest teilweise strukturiertes und/oder oberflächenmodifiziertes Substrat (11, 31, 41, 51, 61, 71) aufgebracht werden und sich adhärent über Bahn-Oberflächenbereiche (13, 15, 17, 35, 45, 55, 57, 77a, 77b) des Substrats unter Erzeugung von Zellspuren (14a, 14b, 34, 44) bewegen, die aus von den Zellen abgetrennten Materialrückständen bestehen, und Zelluntersuchungen an den Zellspuren durchgeführt werden.

2. Verfahren gemäß Anspruch 1, bei dem zur Zelluntersuchung die Menge, die Geometrie, die chemische Zusammensetzung, die passiven elektrischen Parameter und/oder mechanische Eigenschaften der Zellspuren oder von deren Bestandteilen erfaßt werden.

3. Verfahren gemäß Anspruch 2, bei dem zur Erfassung der Menge und Geometrie der Zellspuren Filamente (14a) und Membranflecken (14b) erfaßt werden.

4. Verfahren gemäß Anspruch 2, bei dem zur Erfassung der Zusammensetzung der Zellspuren diese einer Färbung oder Markierung zur Durchführung mikroanalytischer Verfahren unterzogen werden.

5. Verfahren gemäß Anspruch 4, bei dem die mikroanalytischen Verfahren Fluoreszenzmessungen, Messungen auf der Basis von Isotopenmarkierungen oder Elementanalysen umfassen.

6. Verfahren gemäß Anspruch 2, bei dem zur Erfassung der Zusammensetzung der Zellspuren diese einem enzymatischen Abbau unterzogen werden.

7. Verfahren gemäß Anspruch 2, bei dem die Zellspuren mit einem hochauflösenden Mikroskopieverfahren untersucht werden.

8. Verfahren gemäß Anspruch 2, bei dem zytoplasmatische Rückstände oder genetischen Materialien in den Zellspuren erfaßt werden.

9. Verfahren gemäß Anspruch 2, bei dem die Stabilität der Zellspuren bei mechanischen, elektrischen, akustischen, optischen und/oder chemischen Behandlungen erfaßt wird.

10. Verfahren gemäß Anspruch 2, bei dem zur Erfassung der passiven elektrischen Parameter der Zellspuren deren Impedanz, Durchschlagfestigkeit, nichtlineares Verhalten und/oder Erwärmung bei Stromdurchfluß erfaßt werden.

11. Verfahren gemäß Anspruch 2, bei dem zur Erfassung mechanischer Eigenschaften der Zellspuren deren Elastizität oder Plastizität erfaßt werden.

12. Verfahren gemäß einem der vorhergehenden Ansprüche, bei dem eine Vervielfachung von Bestandteilen der Zellspuren zur Erzeugung von Referenzmaterial durchgeführt wird.

13. Verfahren gemäß einem der vorhergehenden Ansprüche, bei dem die Zellspuren in vorbestimmten Bahn-Oberflächenbereichen (13, 15, 17, 35, 45, 55, 57, 77a, 77b) erzeugt werden, die zumindest teilweise zur verstärkten Anhaftung der Zellen mikrostrukturiert und/oder modifiziert sind.

14. Verfahren gemäß einem der vorhergehenden Ansprüche, bei dem die Zellen nach der Erzeugung der Zellspuren einer medizinischen oder meßtechnischen Verwendung, einer Kryokonservierung oder einer weiteren Kultivierung unterzogen werden.

15. Verfahren gemäß einem der vorhergehenden Ansprüche, bei dem auf einer Vielzahl paralleler Bahnen eine Vielzahl von Zellspuren erzeugt und untersucht werden.

16. Verfahren gemäß einem der vorhergehenden Ansprüche, bei dem auf sich kreuzenden Bahnen Zellspuren erzeugt und an Kreuzungsbereichen der sich kreuzenden Bahnen die gegenseitigen Wechselwirkungen der beteiligten Zellen und/oder Zellspuren untersucht werden.

17. Vorrichtung zur zellspurbasierten Untersuchung biologischer Zellen (16, 76a, 76b) mit einem Substrat (11, 31, 41, 51, 61, 71) mit Oberflächenbereichen (12, 52, 72), an denen die Zellen schlechter adhärieren als auf Bahn-Oberflächenbereichen (13, 15, 17, 35, 45, 55, 57, 77a, 77b), in denen die Zellen gut anhaften und sich adhärent bewegen können, wobei die Bahn-Oberflächenbereiche zur Anhaftung von Zellspuren (14a, 14b, 34, 44) eingerichtet sind, die aus von den Zellen abgetrennten Materialrückständen bestehen.

18. Vorrichtung gemäß Anspruch 17, bei der das Substrat in den Oberflächenbereichen (12, 52, 72) und/oder den Bahn-Oberflächenbereichen (13, 15, 17, 35, 45, 55, 57, 77a, 77b) strukturell und/oder chemisch modifiziert ist, um die Anhaftung von Zellspuren zu unterbinden bzw. zu fördern.

19. Vorrichtung gemäß Anspruch 17, bei der das Substrat Teil eines Mikrosystems ist, auf dem die Oberflächenbereiche und die Bahn-Oberflächenbereiche ausgebildet sind, wobei die Bahn-Oberflächenbereiche mindestens eine gerade Bahn bilden.

20. Vorrichtung gemäß einem der Ansprüche 17 bis 19, bei dem das Substrat aus Glas, Silizium oder einem Kunststoff besteht.

21. Vorrichtung gemäß einem der Ansprüche 17 bis 20, bei der eine Vielzahl von Bahn-Oberflächenbereichen in Form einer Gruppe paralleler Bahnen (57) oder sich kreuzender Bahnen (77a, 77b) gebildet sind.

22. Vorrichtung gemäß einem der Ansprüche 17 bis 21, bei dem das Substrat zweiteilig ist, wobei die Bahn-Oberflächenbereiche auf einem der Substratteile angeordnet sind.

23. Verfahren zur zellspurbasierten Kultivierung biologischer Zellen, bei dem die Zellen (16) auf ein zumindest teilweise strukturiertes und/oder oberflächenmodifiziertes Substrat (11) aufgebracht werden und sich adhärent über die Oberfläche des Substrats unter Erzeugung von Zellspuren (14a, 14b) bewegen, die aus von den Zellen abgetrennten Materialrückständen bestehen, und auf den Zellspuren eine Kultivierung von Zellen gleichen oder andersartigen Typs durchgeführt wird.

24. Verfahren gemäß Anspruch 24, bei dem die biologischen Zellen gewebeerzeugende Zellen und das Substrat ein Implantatmaterial umfassen.

25. Verwendung von Materialrückständen, die von biologischen Zellen auf Substraten gebildet sind, zur Untersuchung von Eigenschaften der Zellen für medizinische, biochemische und/oder pharmakologische Zwecke, oder zur biokompatiblen Modifizierung der Oberflächen von Implantatmaterialien.

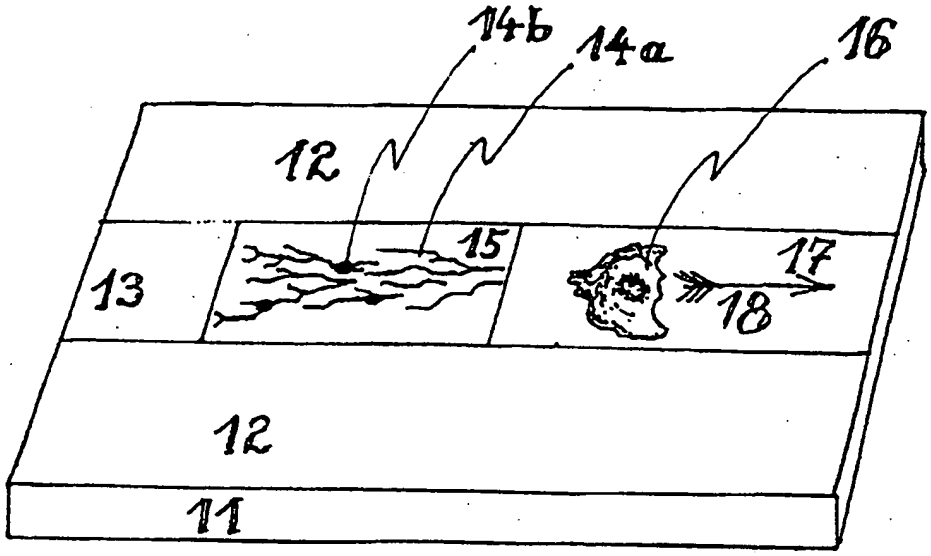

Fig. 1

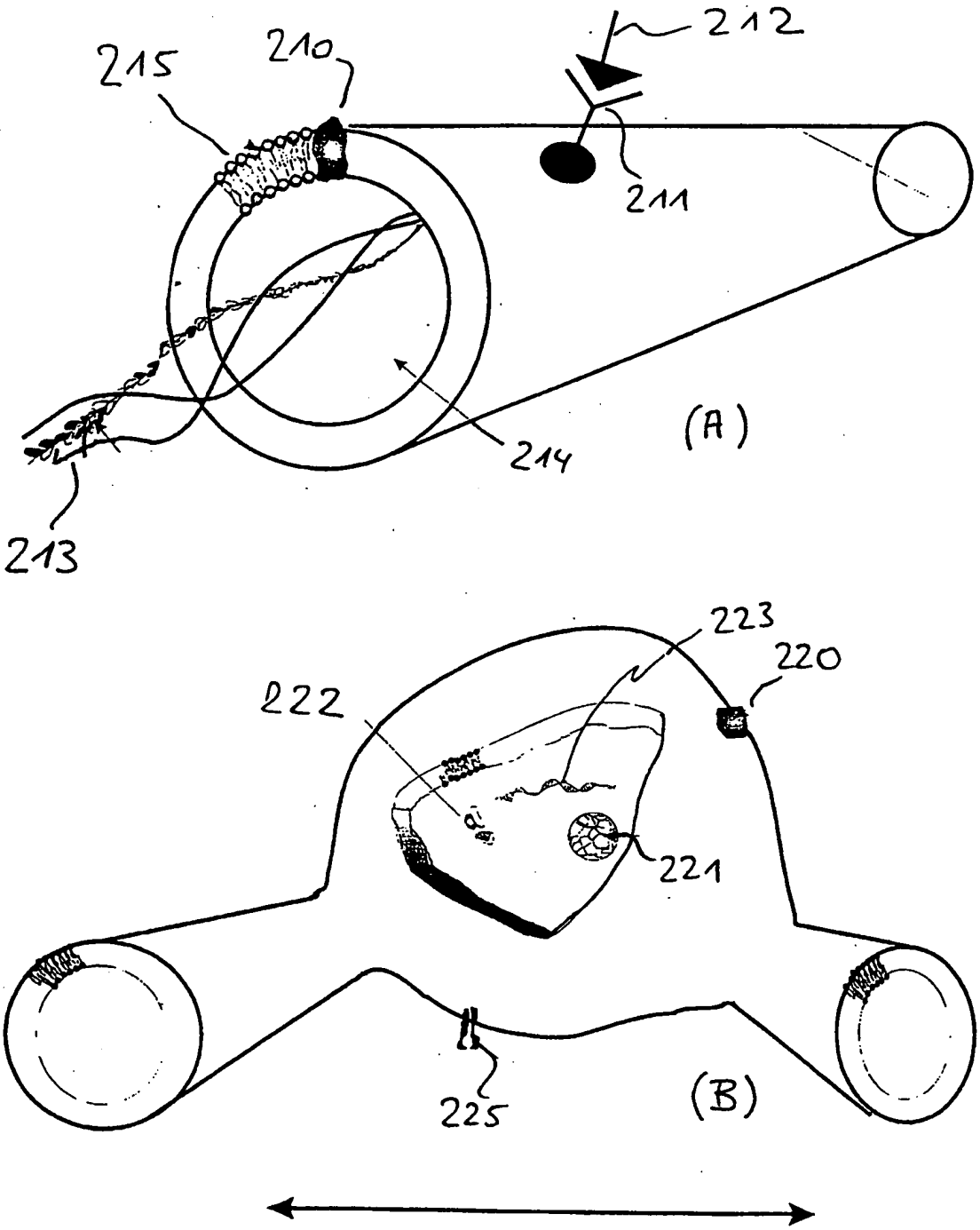

Fig. 2

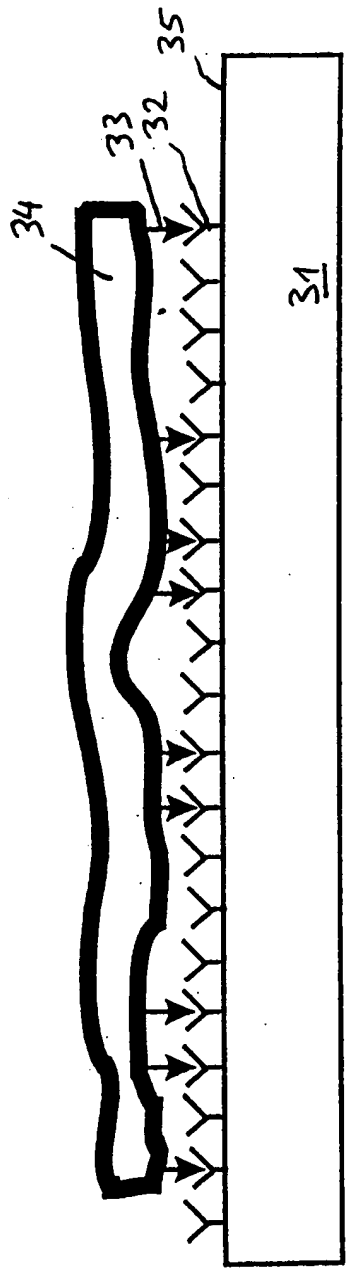

Fig. 3

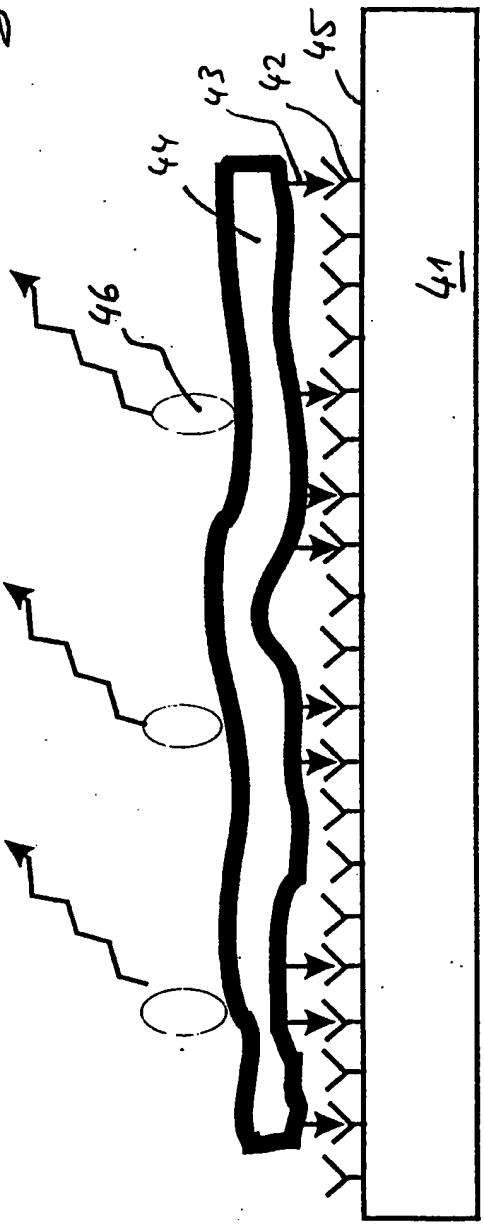

Fig. 4

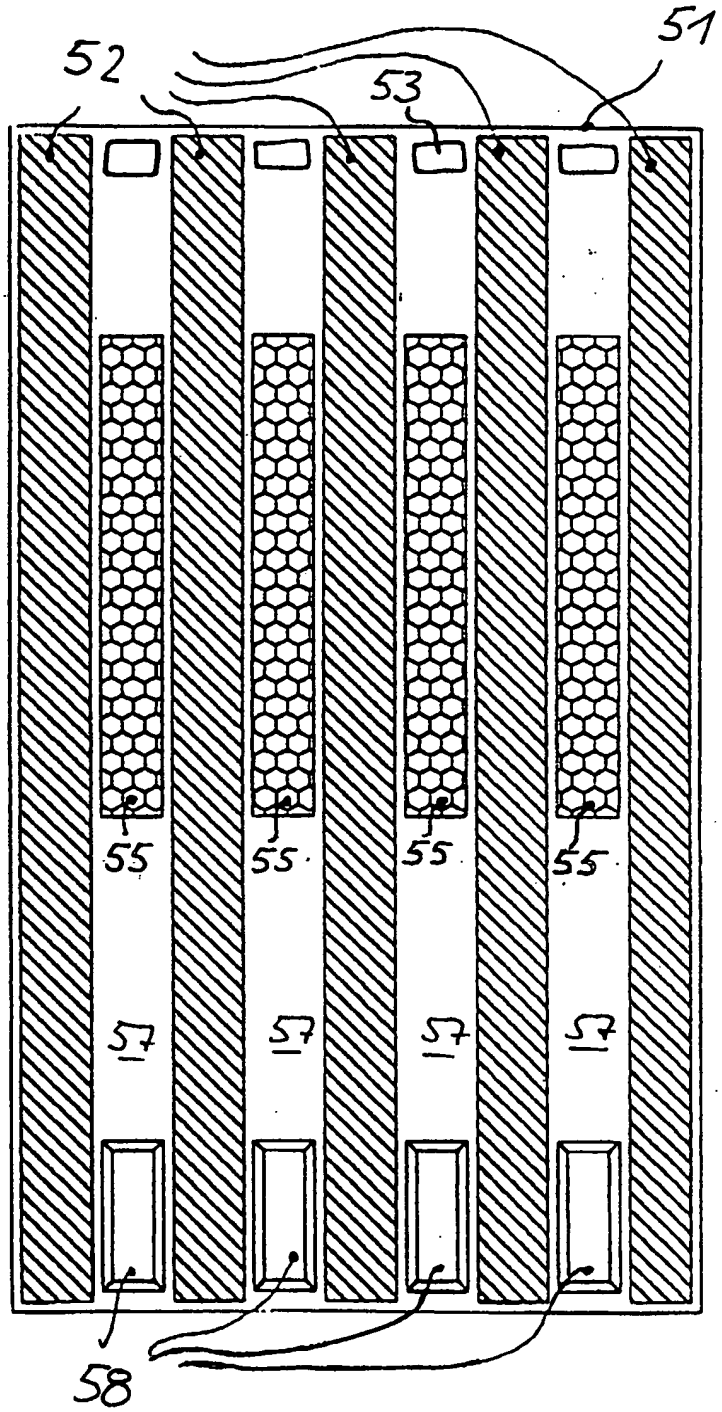

Fig. 5

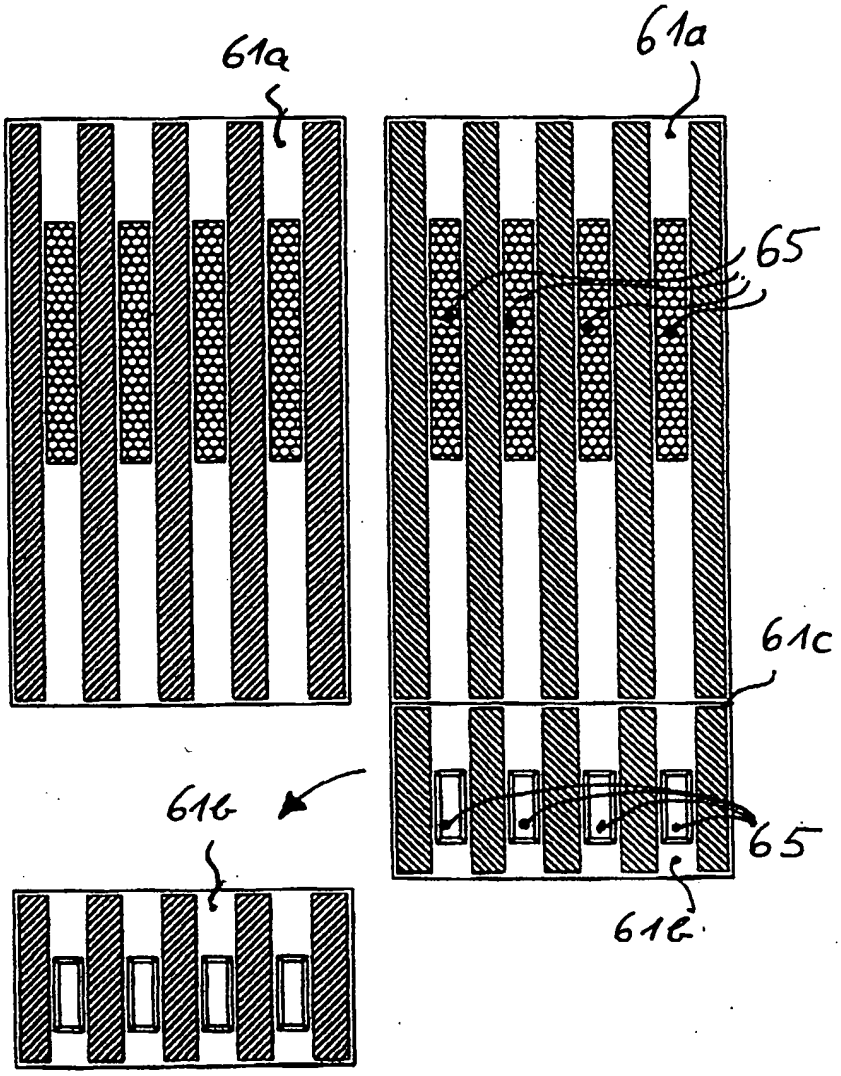

Fig. 6

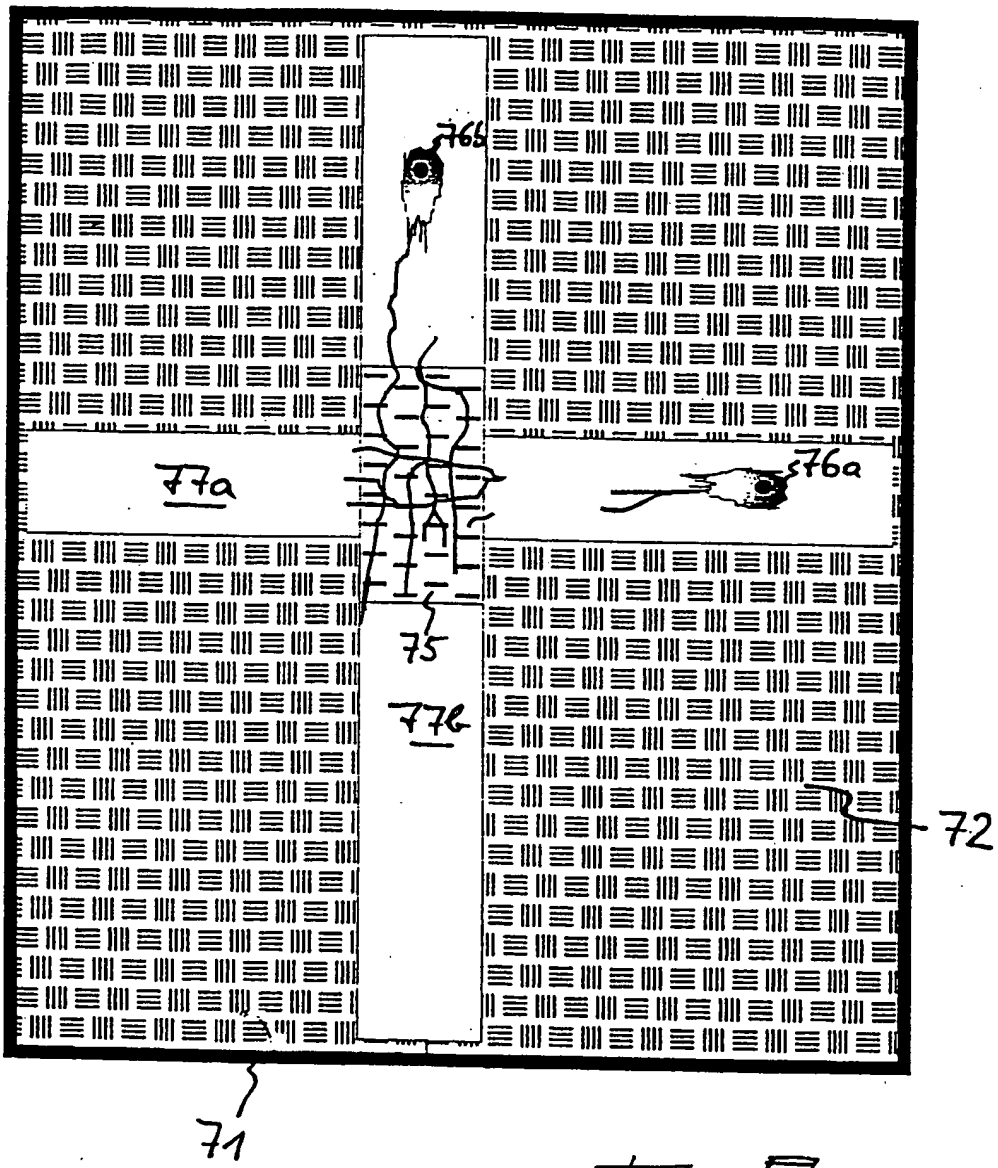

Fig. 7

INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/EP 99/09781

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/483 C12N5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 359 527 A (ZETTER BRUCE R) 16 November 1982 (1982-11-16) column 1, line 64 -column 2, line 8	1,2
Y		4,5,13, 17-20
Y	EP 0 347 210 A (BECTON DICKINSON CO) 20 December 1989 (1989-12-20) page 1, line 52 -page 2, line 4	4,5
X	FR 2 743 421 A (AETSRN) 11 July 1997 (1997-07-11) page 2, line 30 -page 3, line 8 claim 1	1,2,7

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 March 2000	28/03/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Krametz, E

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INTERNATIONAL SEARCH REPORT

Inter. ...nal Application No
PCT/EP 99/09781

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 96 15223 A (UNIV LOUVAIN ;DEWEZ JEAN LUC (BE); LHOEST JEAN BENOIT (BE); DETRAI) 23 May 1996 (1996-05-23) page 3, line 20 -page 7, line 17 figure 1	13,17-20
A	-----	1

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INTERNATIONAL SEARCH REPORT

information on patent family members

Inter onal Application No
PCT/EP 99/09781

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4359527 A	16-11-1982	NONE	
EP 0347210 A	20-12-1989	US 5047321 A	10-09-1991
		AT 111227 T	15-09-1994
		AU 613197 B	25-07-1991
		AU 3596189 A	21-12-1989
		CA 1340170 A	08-12-1998
		DE 68918004 D	13-10-1994
		DE 68918004 T	05-01-1995
		DK 296889 A	16-12-1989
		ES 2063820 T	16-01-1995
		FI 892926 A,B,	16-12-1989
		JP 2009578 C	02-02-1996
		JP 2073157 A	13-03-1990
		JP 7026954 B	29-03-1995
		NO 175506 B	11-07-1994
FR 2743421 A	11-07-1997	AU 1383197 A	01-08-1997
		EP 0879414 A	25-11-1998
		WO 9725617 A	17-07-1997
WO 9615223 A	23-05-1996	BE 1008955 A	01-10-1996
		EP 0800574 A	15-10-1997
		US 5962136 A	05-10-1999

INTERNATIONALER RECHERCHENBERICHT

Inte .onales Aktenzeichen
PCT/EP 99/09781

A. KLASSIFIZIERUNG DES ANMELDUNGSGEGENSTANDES
IPK 7 G01N33/483 C12N5/00

Nach der Internationalen Patentklassifikation (IPK) oder nach der nationalen Klassifikation und der IPK

B. RECHERCHIERTE GEBIETE

Recherchierter Mindestprüfstoff (Klassifikationssystem und Klassifikationssymbole)
IPK 7 G01N C12N

Recherchierte aber nicht zum Mindestprüfstoff gehörende Veröffentlichungen, soweit diese unter die recherchierten Gebiete fallen

Während der internationalen Recherche konsultierte elektronische Datenbank (Name der Datenbank und evtl. verwendete Suchbegriffe)

C. ALS WESENTLICH ANGESEHENE UNTERLAGEN

Kategorie*	Bezeichnung der Veröffentlichung, soweit erforderlich unter Angabe der in Betracht kommenden Teile	Betr. Anspruch Nr.
X	US 4 359 527 A (ZETTER BRUCE R) 16. November 1982 (1982-11-16) Spalte 1, Zeile 64 -Spalte 2, Zeile 8	1,2
Y	---	4,5,13, 17-20
Y	EP 0 347 210 A (BECTON DICKINSON CO) 20. Dezember 1989 (1989-12-20) Seite 1, Zeile 52 -Seite 2, Zeile 4	4,5
X	--- FR 2 743 421 A (AETSRN) 11. Juli 1997 (1997-07-11) Seite 2, Zeile 30 -Seite 3, Zeile 8 Anspruch 1	1,2,7
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[X] Weitere Veröffentlichungen sind der Fortsetzung von Feld C zu entnehmen

[X] Siehe Anhang Patentfamilie

* Besondere Kategorien von angegebenen Veröffentlichungen :

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Datum des Abschlusses der internationalen Recherche	Absendedatum des internationalen Recherchenberichts
21. März 2000	28/03/2000

Name und Postanschrift der Internationalen Recherchenbehörde	Bevollmächtigter Bediensteter
Europäisches Patentamt, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Krametz, E

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INTERNATIONALER RECHERCHENBERICHT

Inte ionales Aktenzeichen
PCT/EP 99/09781

C.(Fortsetzung) ALS WESENTLICH ANGESEHENE UNTERLAGEN

Kategorie°	Bezeichnung der Veröffentlichung, soweit erforderlich unter Angabe der in Betracht kommenden Teile	Betr. Anspruch Nr.
Y	WO 96 15223 A (UNIV LOUVAIN ;DEWEZ JEAN LUC (BE); LHOEST JEAN BENOIT (BE); DETRAI) 23. Mai 1996 (1996-05-23) Seite 3, Zeile 20 -Seite 7, Zeile 17 Abbildung 1	13,17-20
A	------	1

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INTERNATIONALER RECHERCHENBERICHT

Angaben zu Veröffentlichungen, die zur selben Patentfamilie gehören

Internationales Aktenzeichen
PCT/EP 99/09781

Im Recherchenbericht angeführtes Patentdokument		Datum der Veröffentlichung	Mitglied(er) der Patentfamilie		Datum der Veröffentlichung
US 4359527	A	16-11-1982	KEINE		
EP 0347210	A	20-12-1989	US	5047321 A	10-09-1991
			AT	111227 T	15-09-1994
			AU	613197 B	25-07-1991
			AU	3596189 A	21-12-1989
			CA	1340170 A	08-12-1998
			DE	68918004 D	13-10-1994
			DE	68918004 T	05-01-1995
			DK	296889 A	16-12-1989
			ES	2063820 T	16-01-1995
			FI	892926 A,B,	16-12-1989
			JP	2009578 C	02-02-1996
			JP	2073157 A	13-03-1990
			JP	7026954 B	29-03-1995
			NO	175506 B	11-07-1994
FR 2743421	A	11-07-1997	AU	1383197 A	01-08-1997
			EP	0879414 A	25-11-1998
			WO	9725617 A	17-07-1997
WO 9615223	A	23-05-1996	BE	1008955 A	01-10-1996
			EP	0800574 A	15-10-1997
			US	5962136 A	05-10-1999